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Reproductive behaviour of the European Common Frog (*Rana temporaria*)

Das Reproduktionsverhalten des Grasfrosches (*Rana temporaria*)

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Dipl. Biol. Carolin Dittrich

Präsidentin der Humboldt Universität zu Berlin
Prof. Dr.-Ing. Dr. Sabine Kunst

Dekan der Lebenswissenschaftlichen Fakultät der Humboldt-Universität zu Berlin
Prof. Dr. Bernhard Grimm

Gutachter/innen

1. PD Dr. Mark-Oliver Rödel
2. Prof. Dr. Gerlind Lehmann
3. PD Dr. Mirjam Knörnschild
4. Prof. Dr. John Nyakatura

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Abstract

The search and competition for mating partners lead to the evolution of various mating systems, strategies and tactics to increase lifetime reproductive success. The mating behaviour is influenced by natural and sexual selection, whereby both could act in different directions. For most individuals, survival is essential in order to reproduce as often as possible to increase lifetime reproductive fitness. On the other hand, reproduction could increase predation risk due to conspicuous behaviour and risks associated with mating itself. Sexual selection could favour specific secondary sexual traits, either due to advantages in intrasexual competition, or by specific preferences of the choosy sex (intersexual selection). For mate choice to evolve, there need to be benefits associated with the chosen mating partner, because choosiness involves costs in terms of energy and time constraints during mating. As an explosive breeder, the European Common Frog (*Rana temporaria*) has to deal with time constraints during the short breeding season. The males are competing for the access to females and it is assumed that females are passive during breeding due to a high male-biased operational sex ratio. However, from an evolutionary perspective females should be the choosy sex and should decide with whom to mate, as they invest more energy into the production of eggs.

In my thesis, I examine the mating and reproductive behaviour of the European Common Frog in an evolutionary context. I aim to understand which mechanisms lead to the formation of pairs, if mate choice shapes the patterns of mating that we can observe and if there are benefits derived from pairing with a specific mate.

In paper 1 I show that size-assortative mating exists in different populations over consecutive years. The formation of size assorted pairs is partly due to a large male advantage during scramble competition, seen as a higher probability of large males being paired. Furthermore, I observed temporal covariation in migration patterns; larger individuals of both sexes arrive earlier at the breeding sites, which increases the probability for size assortment. Although size assortment should benefit fertilisation success, I could not detect differences in number of eggs fertilised after pairing individuals of differing body sizes. Nevertheless, smaller males seem to be faster when grabbing a female, which indicates prudent male choice. Together with a higher probability of larger females being paired, these findings indicate mate choice behaviour.

Paper 2 describes two different female release calls that were emitted when grabbed by a male. Although these two calls are similar in structure, they differ in their dominant frequencies and bandwidth of frequency. One call seems to imitate the male release call, as they share the same dominant frequency and this call is emitted by males when grabbed by another male. This call is termed the grunting sound. The second call, termed squeaking sound, shows a higher dominant frequency and larger bandwidth. Therefore, it seems to be a distress call that could be emitted against rather unspecific receivers.

In paper 3 I investigate mate choice behaviour during pair formation. Males do not show mate choice behaviour considering female body size and have a high failure rate when trying to clasp a female. The females though show three different behaviours to probably test the strength and endurance of the male. The most common behaviour was rotation of the females body, which could be seen in 83% of all females. The most astonishing behaviour observed was death feigning, which was shown in 33% of all females. Additionally, two different release calls could be recorded (paper 2). All of the three behaviours seem to be associated with fight-or-flight stress responses. Females are prone to drowning during mating, if too many males could get hold to her. Therefore, it could be beneficial if the female chooses a strong male who protects her from the formation of so called "mating-balls". This choice would increase her survival chances and therfore lifetime reproductive fitness.

In the fourth paper I investigated the genetic effects of parents on developmental traits in their offspring. I could show that multiple paternity is common in *Rana temporaria* and that additive genetic effects of fathers are increasing the variability of developmental time of their offspring. This increase in variability could be beneficial in uncertain environments like the

temporal ponds where Common Frogs are breeding.

I could show that studying mating behaviour in a common species can shed light on the evolution of behavioural traits. In this species we find mate choice behaviour displayed by females, which could increase lifetime fitness due to an increase in survival chances when finding a good mate to protect her from the formation of mating balls. Survival of the female to the next breeding season could be the evolutionary benefit of female mate choice. This theory is supported by the high rates of multiple paternity. Females cannot influence paternity after deposition of clutches and therefore indirect benefits of mate choice could be negligible. Male mate choice seems to be a costly trait and alternative mating tactics have evolved, as seen in the large male advantage and indiscriminate mate choice.

Kurzfassung

Die Suche nach und die Konkurrenz um Paarungspartner führt zur Entwicklung verschiedener Paarungssysteme, Strategien und Taktiken, um den Reproduktionserfolg während der gesamten Lebensdauer zu erhöhen. Das Paarungsverhalten wird durch natürliche und sexuelle Selektion beeinflusst, wobei beide in unterschiedliche Richtungen wirken können. Für die meisten Individuen ist das Überleben unerlässlich, um sich so oft wie möglich zu reproduzieren, und dadurch die reproduktive Gesamtfitness zu erhöhen. Andererseits könnte ein auffälliges Verhalten bei der Fortpflanzung das Prädationsrisiko erhöhen. Der Akt der Paarung selbst kann bereits mit Risiken verbunden sein, welche sich auf die Überlebensraten auswirken können. Durch sexuelle Selektion könnten bestimmte sekundäre Geschlechtsmerkmale begünstigt werden, entweder aufgrund von Vorteilen im Wettbewerb innerhalb eines Geschlechts (intrasexuell), oder aufgrund spezifischer Präferenzen zwischen den Geschlechtern (intersexuelle Selektion). Damit sich die Partnerwahl entwickeln kann, muss der gewählte Paarungspartner Vorteile aufweisen, von denen der wählende Partner profitiert, denn die Wahl ist mit energetischen Kosten und zeitlichem Aufwand verbunden. Als Frühlaicher muss der Europäische Grasfrosch (*Rana temporaria*) mit einem eingeschränktem Paarungszeitraum umgehen. Die Männchen konkurrieren um den Zugang zu Weibchen und es wird angenommen, dass sich Weibchen während der Paarung und Reproduktion passiv verhalten, da der hohe "Männchen-Überschuss" keine Wahl zulassen würde. Aus evolutionärer Sicht sollten Weibchen jedoch das wählerische Geschlecht sein und entscheiden mit wem sie sich paaren, da sie mehr Energie in die Eierproduktion investieren.

In meiner Dissertation untersuche ich das Paarungs- und Fortpflanzungsverhalten des Europäischen Grasfrosches in einem evolutionären Kontext. Mein Ziel ist es zu verstehen, welche Mechanismen zur Bildung von Paaren führen, ob die Partnerwahl die Paarungsmuster erklärt, die wir beobachten können, und ob es evolutive Vorteile gibt, die sich aus der Paarung mit einem bestimmten Partner ergeben.

In Manuskript 1 zeige ich, dass in verschiedenen Populationen über mehrere Jahre hinweg, eine größensortierte Paarung zu beobachten ist. Die Bildung von größensortierten Paaren ist zum Teil auf den Vorteil größerer Männchen im Wettstreit um Weibchen zurückzuführen, was dadurch bestätigt wird, dass größere Männchen mit einer höheren Wahrscheinlichkeit verpaart sind Zusätzlich beobachtete ich zeitliche Kovariationen in den Migrationsmustern; größere Individuen beider Geschlechter kommen früher an den Fortpflanzungsgewässern an, was eine größensortierte Paarung begünstigt. Entgegen der Annahme, dass eine Größensortierung den Befruchtungserfolg erhöhen sollte, konnte ich keine Unterschiede in der Anzahl der befruchteten Eier feststellen, nachdem ich Individuen verschiedener Körpergrößen verpaarte. Nichtsdestotrotz scheinen kleinere Männchen beim Ergreifen eines Weibchens schneller zu sein, was auf eine umsichtige männliche Wahl hinweist. Zusammen mit der höheren Wahrscheinlichkeit, dass sich größere Weibchen in einer Verpaarung befinden, deuten diese Ergebnisse auf ein Partnerwahlverhalten hin.

Manuskript 2 beschreibt zwei verschiedene weibliche Befreiungsrufe, die beim Ergreifen durch ein Männchen ausgelöst wurden. Obwohl diese beiden Rufe in ihrer Struktur ähnlich sind, unterscheiden sie sich in ihren dominanten Frequenzen und in der Bandbreite des abgedeckten Frequenzbereichs. Einer der weiblichen Befreiungsrufe scheint den männlichen Befreiungsruf nachzuahmen, da beide Rufe die gleiche dominante Frequenz besitzen. Dieser Ruf wird als grunzender Ruf bezeichnet. Der zweite Ruf, das so genannte "quietschen", zeigt eine höhere dominante Frequenz und eine größere Bandbreite des Frequenzbereichs. Es könnte sich um einen Befreiungsruf handeln, der für eher unspezifische Empfänger verwendet wird.

In Manuskript 3 untersuche ich das Partnerwahlverhalten während der Paarbildung. Männchen zeigen keine Partnerwahl hinsichtlich der Körpergröße von Weibchen und haben eine hohe Fehlerrate bei dem Versuch ein Weibchen zu ergreifen. Die Weibchen zeigen jedoch drei verschiedene Verhaltensweisen, vermutlich um die Stärke und Ausdauer der Männchen zu testen.

Das am häufigsten beobachtete Verhalten war die Rotation des weiblichen Körpers, welche bei 83% aller Weibchen zu beobachten war. Das erstaunlichste Verhalten war das Vortäuschen des Todes, das von 33% aller Weibchen gezeigt wurde. Zusätzlich konnten zwei verschiedene Befreiungsrufe aufgezeichnet werden (Manuskript 2). Alle drei Verhaltensweisen scheinen im Zusammenhang mit Stressreaktionen bei Kampf- oder Flucht-Reaktionen zu stehen. Weibchen sind anfällig für das Ertrinken während der Paarung, wenn sich zu viele Männchen an ihr festhalten. Daher könnte es von Vorteil sein, sich für ein starkes Männchen zu entscheiden, das sie vor der Bildung so genannter "Mating-Balls" schützt. Dieses Wahlverhalten könnte die Überlebenswahrscheinlichkeit des Weibchens erhöhen und somit auch ihre reproduktive Fitness.

Im vierten, und damit letzten Manuskript, untersuche ich die genetischen Auswirkungen von Eltern auf die Entwicklungsmerkmale ihrer Nachkommen. Ich konnte zeigen, dass multiple Vaterschaft in *Rana temporaria* häufig auftritt und, dass additive genetische Effekte von Vätern die Variabilität in der Entwicklungszeit von Nachkommen erhöhen. Diese Zunahme der Variabilität könnte in unsicheren Umgebungen, wie den temporären Gewässern in denen Grasfrösche laichen, von Vorteil sein.

Ich konnte zeigen, dass Untersuchungen des Paarungsverhaltens einer häufigen Art, Licht auf die Entwicklung von Verhaltensmerkmalen werfen können. Weibchen dieser Art weisen Partnerwahlverhalten auf, welches die Überlebenschancen steigert und somit die Lebenszeitfitness erhöhen könnte. Ein Überleben bis zur nächsten Fortpflanzungssaison könnte der evolutive Vorteil sein, der durch die Partnerwahl entsteht. Diese Theorie wird gestützt durch die hohen Raten der mehrfachen Vaterschaft. Weibchen haben nach dem Ablaichen keinen Einfluss auf die Vaterschaft ihrer Nachkommen und ein indirekter Vorteil durch Partnerwahl entfällt. Eine Partnerwahl durch Männchen scheint ein kostspieliges Merkmal zu sein und es haben sich hierzu alternative Paarungstaktiken entwickelt, was sich aus dem Paarungsvorteil großer Männchen und der willkürlichen Partnerwahl schließen lässt.

Preface and author's contributions

This thesis contains a general introduction and a synthesis section that recapitulates the most important findings from the papers. The results of this thesis are presented in four scientific papers of which two are published, one is submitted and the last is in preparation for submission. The contributions of the co-authors to each paper are listed below. From all four papers, the PhD candidate (C. Dittrich) is the first author.

The papers tackle the following topics:

Paper 1
Dittrich, C., Rodríguez, A., Segev, O., Drakulić, S., Feldhaar, H., Vences, M., & Rödel, M.-O. (2018). Temporal migration patterns and mating tactics influence size-assortative mating in *Rana temporaria*. Behavioral Ecology, 29, 418–428.
Author's Contribution: designed study: C. Dittrich (partial), H. Feldhaar, M. Vences, A. Rodríguez, M.-O. Rödel; collected field and experimental data: C. Dittrich (large), A. Rodríguez, O. Segev, H. Feldhaar, S. Drakulić , M. Vences, M.-O. Rödel; analyzed data: C. Dittrich (large), A. Rodríguez , O.Segev; drafted first version of the manuscript: C. Dittrich (large), A.Rodríguez. All authors read, commented on, and approved the final version of the manuscript

Paper 2
Dittrich, C., & Rödel, M.-O. (2020). Description of female release calls of the European Common Frog, *Rana temporaria* (Anura: Ranidae). Salamandra, 56, 91–94.
Author's Contribution: designed study: C. Dittrich (large), M.-O. Rödel; collected field data and experimental data: C. Dittrich (large), M.-O. Rödel; analysed data: C. Dittrich; drafted first version of the manuscript: C. Dittrich; commented on, and approved the final version of the manuscript: M.-O. Rödel

Paper 3
Dittrich, C., & Rödel, M.-O. (in prep.). The freedom of choice – Female mate choice behaviour in *Rana temporaria*
Author's Contribution: designed study: C.Dittrich (large), M.-O. Rödel; collected field data and experimental data: C. Dittrich (large), M.-O. Rödel; analyzed data: C. Dittrich; drafted first version of the manuscript: C. Dittrich; commented on, and approved the final version of the manuscript: M.-O. Rödel

Paper 4
Dittrich, C., Huster, J., Rödel, M.-O., & Feldhaar, H. (2019). Matriline effects on metamorphic traits in a natural system in the European common frog (*Rana temporaria*). Ecology & Evolution, 9, 3075–3088.
Author's Contribution: designed study: J. Huster, H. Feldhaar, M.-O. Rödel; collected field data and experimental data: J. Huster; analyzed data: C. Dittrich (large), J. Huster; drafted first version of the manuscript: C. Dittrich; commented on, and approved the final version of the manuscript: M.-O. Rödel, H. Feldhaar, J. Huster

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The field work was done in one of the most rural areas from Franconia and I want to thank former and current members of the ecological field station Fabrikchleichach (University Würzburg) for their friendly and warm welcoming each year that I spend there. I really liked to be part of their team.

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Contents

1 Introduction

The world's ecosystems are constantly changing, which can have tremendous effects on the organisms living there. The recent wave of species extinctions is considered the sixth mass extinction in the long history of evolution. Species extinction rates in the last century have been higher than the usual background extinction rates over the last million years (Ceballos et al., 2015). Additionally, climate change rates are higher than the evolution of climate niche breath in most vertebrate species (Quintero and Wiens, 2013) and could lead to further range size contractions. Although species with small range sizes and small population sizes are at higher risk of extinction, species that are widespread and of least concern, according to the IUCN Red List categories, are dropping in population numbers and range sizes (Ceballos et al., 2017). This loss of biodiversity could have severe impacts on ecosystem functioning (Naeem et al., 2012).

Amphibians are one of the most threatened vertebrate groups worldwide (Stuart et al., 2004; Collins, 2010; Alroy, 2015). Especially amphibians occurring at higher latitudes exhibit proportionally more decreasing species, contrary to other vertebrate groups like birds (Fig. 1) (Ceballos et al., 2017). They react sensitive and fast to environmental changes due to their complex life cycle (Wilbur, 1980; Kiesecker et al., 2001), their semi-permeable skin (Quaranta et al., 2009) and their usually specific habitat requirements (Lemckert, 2004; Baldwin et al., 2006). The main factors of amphibian decline are habitat changes and fragmentation of landscapes (Funk et al., 2005). Additional stressors that influence amphibian populations are climat change (Araújo et al., 2006; Case et al., 2015), ongoing pollution with pesticides (Brühl et al., 2013), the spread of amphibian diseases (Martel et al., 2014; Van Rooij et al., 2015) and over-exploitation (Stuart et al., 2004).

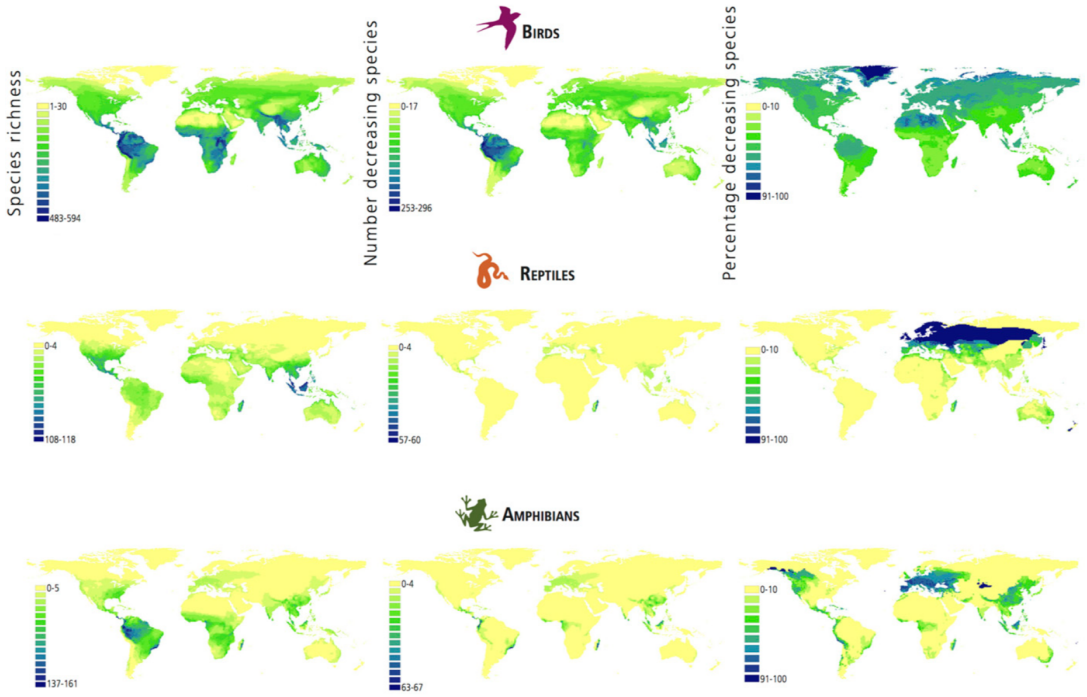

Figure 1 Worldwide species richness (left), number of decreasing species (middle) and percentage of decreasing species for birds, reptiles and amphibians. Especially amphibians in higher latitudes show proportionally higher declines than birds. Figure taken from Ceballos et al., 2017.

Successful reproduction will be the main force for populations to survive and persist. To reproduce successfully individuals need to find a mate of their own species and ideally this mate should be of good quality. In order to present good quality to potential mating partners and to detect these qualities, several different behaviours and sexual dimorphism evolved by

sexual selection. Sexual selection is based on processes that increase the reproductive fitness of individuals, thereby influencing mating probabilities, where some individuals with specific traits will have higher mating probabilities than others. There is general consent that females are the choosy sex in most species, due to their high investment in eggs (Trivers, 1972). However, choosiness is associated with costs, such as search time for a mate, the energy invested in searching and an increase in predation risk due to conspicuous behaviour of the chooser (Jennions and Petrie, 1997). Therefore, for choosiness to evolve there need to be benefits, which outweigh the costs. These benefits could be either direct, e.g. gain of resources, provision of parental care or higher survival chances, or indirect, e.g. increase of offspring survival due to genetic effects (compatibility, good genes).

Amphibians show different mating and breeding systems in which female temporal availability is one of the main factors that shape these systems (Wells, 2007). Other factors are seasonal patterns of water availability and biotic factors like predation risk and competition (Wells, 2007). The main mating systems are a) choruses and leks, b) resource defences and c) scramble competition (Wells, 2007). Additionally, depending on operational sex ratio and mode of fertilization (Zamudio and Chan, 2008), alternative mating tactics are common, e.g. satellite males (Arak, 1983) or clutch piracy (Fig. 2) (Vieites et al., 2004).

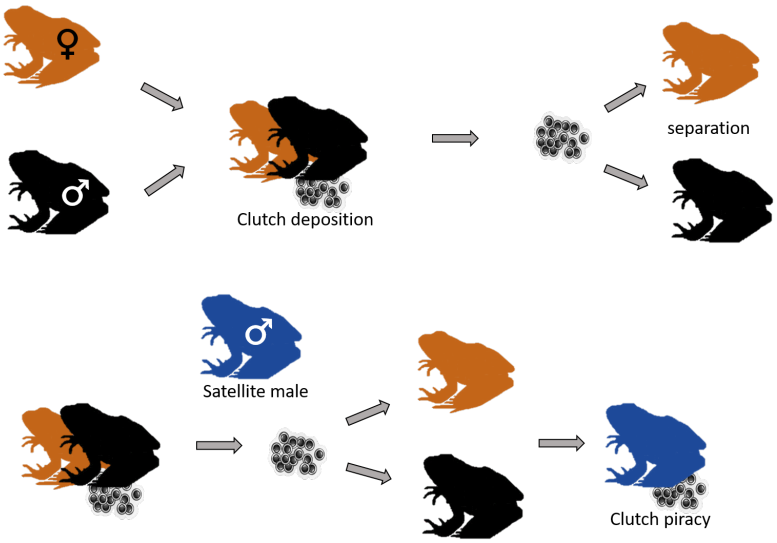

Figure 2 Schematic presentation of usual *Rana temporaria* egg clutch fertilisation, the pair separates after egg deposition (upper panel) and post-mating clutch piracy by an additional satellite male could occur (lower panel), modified figure after Vieites et al., 2004.

The European Common Frog (*Rana temporaria* Linneaus, 1758) is one of the most widespread amphibians worldwide, which inhabits a broad variety of different habitats in Europe (Günther, 1996; Gollmann et al., 2014), with a preference towards forested areas with dense herbaceous vegetation and temperate climate (Blab, 1978; Marnell, 1998). The species thus has a high ecological amplitude and we consider it as highly plastic in habitat requirements and its adaptation potential. Nevertheless, populations are decreasing in this generalist species. Their reproduction takes place in large breeding aggregations in early spring within a short period of time (Günther, 1996; Gollmann et al., 2014) and tadpoles show high phenotypic plasticity in their developmental traits (Lindgren and Laurila, 2010; Grözinger et al., 2014, 2018). Therefore, the European Common Frog is an excellent model organism to study breeding and mating behaviour, the influence of mate choice on size-assortative mating and interactions of genetics provided by the parents.

2 Synthesis

2.1 Size-assortative mating

Assortative mating is a widespread pattern in sexually reproducing species, but the mechanisms that lay behind that assortment remain insufficiently understood. In amphibians size-assortative mating was found in a variety of taxa (Davies and Halliday, 1977; Robertson, 1990), but is considered to be a secondary sorting due to large male advantage during scramble competition (Green, 2019). I show that the European Common Frog is exhibiting size-assortative mating in high and low competition scenarios (Fig. 3). In low competition scenarios temporal migration patterns of differently sized individuals migrating to the pond support size assortment, because larger individuals arrive first at the breeding site. During scramble competition, large males have a higher probability of being paired, because they are stronger than their smaller conspecifics and could take over already paired females, although smaller males are faster in grabbing a female. Additionally, larger females had a higher probability of being paired independent from male densities. This is an indicator for male mate choice, which I tested in the following chapters while excluding male competition (paper 2 and 3). I show that size-assortment has no influence on fertilisation success and therefore does not seem to have an indirect benefit on number of offspring, although it might influence quality of offspring, which is partly covered in paper 4.

Figure 3 Size-assorted pairs of *Rana temporaria* found during migration to the breeding sites at 23.03.19, Fabrikschleichach, lower Franconia, Germany.

2.2 Release calls

Anuran communication research is mainly based on studies about advertisement calls emitted by males. They serve as a conspecific signal for potential mating partners and are employed as species identifier for taxonomy and bioacoustics surveys (Köhler et al., 2017). In this study (paper 2), I describe two distinct calls of *Rana temporaria* females, which were emitted during the mate choice experiments (paper 3), when the male was grabbing the female. These two calls might have different meanings for the receiver. The first sound seems to imitate the male release call as it contains the same main dominant frequency, and could therefore act repulsive to the male (referred to as grunting sound). As shown in paper 3 the application of these call type lead to a higher escape rate of females. The second sound contains a higher dominant frequency with a broader frequency range, which is a typical property of unspecific distress calls (referred to as squeaking sound, Fig. 4). Distress calls are not addressed to a specific receiver and should lead

to the confusion of predators and is therefore a defence mechanism against predation (Toledo et al., 2015). The application of this call type was less successful considering escape from male amplexus. Release or distress calls did not receive much attention so far, probably of the rather unspecific signals given to a receiver. However, both of these calls can be seen as mate choice mechanisms of the females.

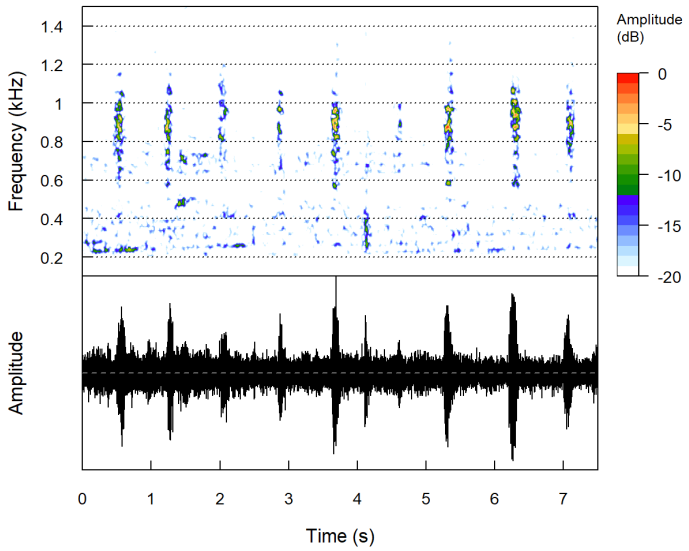

Figure 4 Spectrogram and oscillogramm of *Rana temporaria* female ID27 (SVL: 58 mm; pair 17; TSA: Rana_temporaria_DIG0204_07) emitting squeaking sound after being grabbed by a male. Dominant frequency; average ± SD: 914 ± 53 Hz; bandwidth: 550–1148 Hz. Figure taken from paper 3.

2.3 Mate choice

After Darwins theory of sexual selection there are two main rules. Number one is to find a mate of the same species and number two to find a "good" mate (Darwin, 1871). Mate choice should occur when the chooser gains benefits from being choosy, as choosiness involves energy and time constraints (Jennions and Petrie, 1997). Until now, it has been believed that in most explosive breeding species, in which large breeding aggregations form within a short time period; males are the choosy sex and compete in scramble competition for access to females (Fig. 5). In my first study, I show that large males have an advantage in scramble competition, but also that larger females have a higher probability of being paired. The freedom of mate choice for females is mainly neglected and passiveness is an assigned trait. However, some studies in other temperate explosive breeders did show that females have strategies to avoid unwanted mating (Hettyey et al., 2005, 2009). In this study (paper 3), I demonstrate that *Rana temporaria* males are mating randomly and do not show a mating preference based on female body size. More importantly, I revealed that female Common Frogs do exhibit active mate choice by either rotating their bodies, emitting release calls or feigning death, probably in order to test the strength or endurance of a potential mating partner. The application of one or several of these behaviours lead to an escape rate of 50%. Additionally, females smaller than males have higher escape probabilities and do exhibit mate choice more often. I herein could demonstrate that females in explosive breeding species are not passive and do have the freedom to choose their mating partner, contrary to the common opinion in the literature.

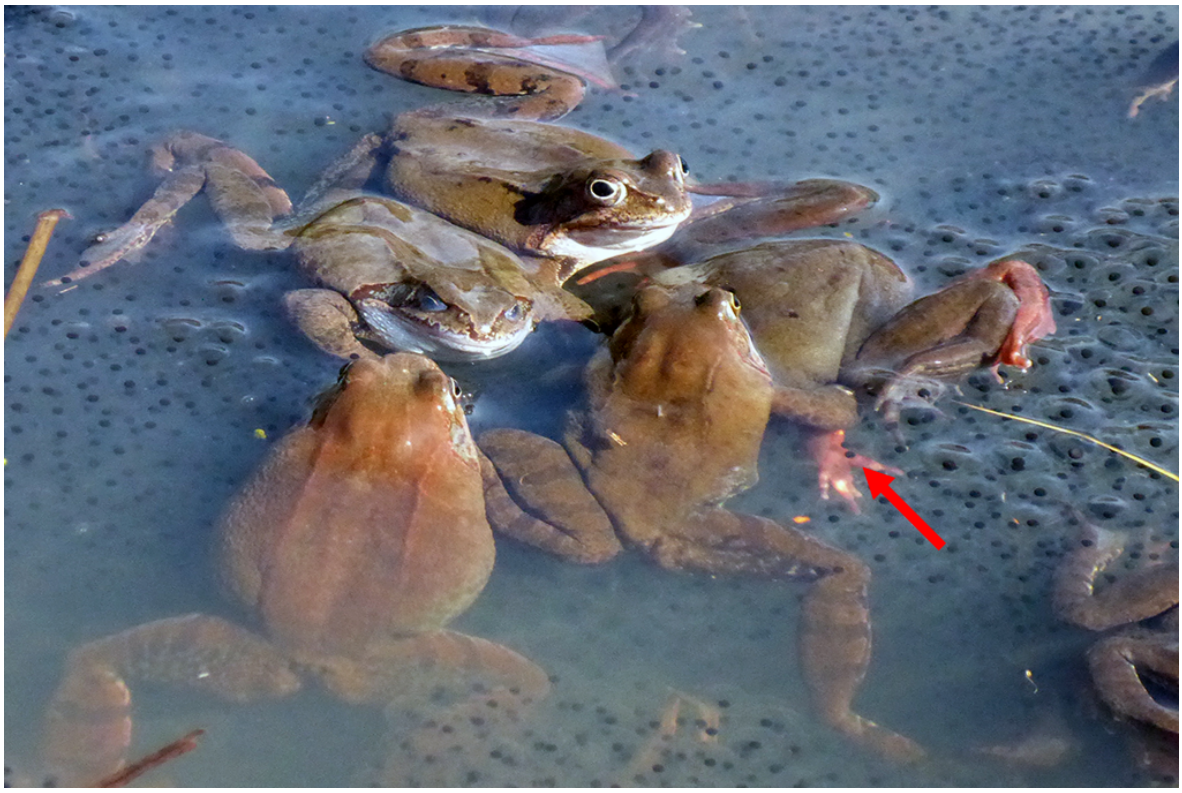

Figure 5 Breeding aggregation of the European Common Frog (*Rana temporaria*) with male biased operational sex ratio. The red arrow points towards a female frog, which is surrounded by males. Fabrikschleichach, lower Franconia, Germany, 25.03.2017.

2.4 Matriline effects on developmental traits

Metamorphosis is the most important life history process experienced by animals with complex life cycles (Wilbur, 1980). The timing and outcome of this event has crucial implications for the individual considering further development and life history (Chelgren et al., 2006). Offspring of the European Common Frog show high phenotypic plasticity that cannot be explained by environmental factors alone (Grözinger et al., 2014, 2018). There needs to be an underlying variability that is not determined by environmental factors. Therefore, I determine the genetic influence of specific mothers (matrilines) and the effect of multiple paternity on developmental traits of their offspring (paper 4). Most studies so far were conducted in a laboratory mesocosm setting, in which only single or a few environmental factors have been altered. The novelty of this study is the usage of multilocus, polymorphic microsatellite data to assign metamorphs to their respective mothers (matriline) in a natural setting. This natural setting allowed me to identify developmental traits, which are more strongly influenced by the environment (body size and body condition) and those that are more strongly influenced by additive genetic effects of fathers (developmental time). I found that in 80% of all deposited clutches multiple paternity occurred(Fig. 6) resulting in an increased variability of developmental traits.

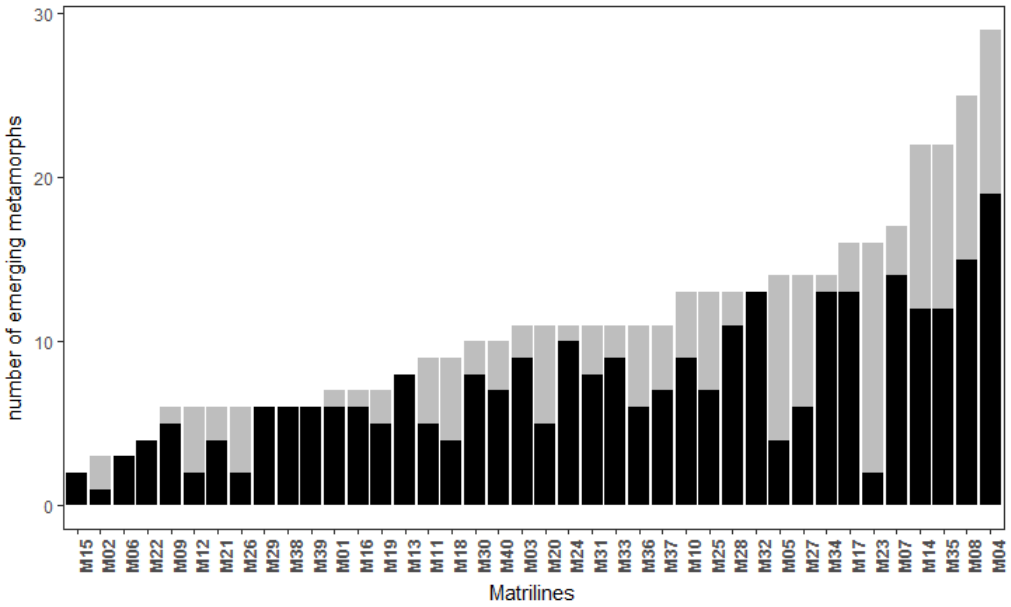

Figure 6 Number of emerging metamorphs per matriline from main father (black, n= 294) and all fathers (grey, n= 439). In 80% of all matrilines we could find multiple paternity. Figure taken from Dittrich et al. 2019.

3 Conclusion

My thesis extents the knowledge of the breeding and mate choice behaviour of a (temporary) common species and reveals that size-assortative mating arises from a mixture of temporal availability of mates and secondary sorting during scramble competition of males. Size assortment has no influence on fertilisation success and therefore does not seem to have fitness benefits. Despite the fact that larger females show a higher probability of being paired, males do not exhibit mate choice behaviour based on female body size alone and are mating randomly. Females seem to be the ones that choose their mating partners by applying three different mate choice behaviours,i.e. rotating, calling and feigning death. They might test the strength of the male or his endurance, thereby trying to avoid inferior partners that could not protect her from being grabbed by several males, which could lead to her death by drowning when trapped in a "mating-ball". Females do not seem to be silent since they communicate acoustically to reject potential mates or to escape inferior ones. These kind of communication in anurans has received little attention so far. Finally, multiple paternity seems to be quiet common in *Rana temporaria* and additive genetic effects of fathers can add variability to offspring's developmental traits, thereby increasing their survival chances in a temporary unstable environment. Due to the prevailing multiple paternity, sexual selection in terms of mate choice should have minor effects on this species and the behaviours exhibited by the females could be seen as survival strategies to make it to the next breeding season and increase lifetime reproductive fitness. Therefore, surviving the mating season seems to be the most important selection mechanism in this species.

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5 Papers

Paper 1

Temporal migration patterns and mating tactics influence size-assortative mating in *Rana temporaria*.

Dittrich, C., Rodríguez, A., Segev, O., Drakulić, S., Feldhaar, H., Vences, M., & Rödel, M.-O. (2018). Temporal migration patterns and mating tactics influence size-assortative mating in *Rana temporaria*. Behavioral Ecology, 29(2), 418–428. doi:10.1093/beheco/arx188. Published 10 January 2018

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Original Article

Temporal migration patterns and mating tactics influence size-assortative mating in *Rana temporaria*

Carolin Dittrich,[a] Ariel Rodríguez,[b,c] Ori Segev,[d] Sanja Drakulić,[a] Heike Feldhaar,[e] Miguel Vences,[b] and Mark-Oliver Rödel[a,f]

[a]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Invalidenstr. 43, 10115 Berlin, Germany, [b]TU Braunschweig, Zoologisches Institut, Evolutionsbiologie, Mendelsohnstraße 4, 38106 Braunschweig, Germany, [c]University of Veterinary Medicine Hannover, Institute of Zoology, Bünteweg 17, 30559 Hannover, Germany, [d]University of Haifa, Faculty of Science, Institute of Evolution, Community Ecology Lab, Haifa 31905, Israel, [e]University of Bayreuth, Animal Population Ecology, 95440 Bayreuth, Germany, and [f]Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

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Assortative mating is a common pattern in sexually reproducing species, but the mechanisms leading to assortment remain poorly understood. By using the European common frog (*Rana temporaria*) as a model, we aim to understand the mechanisms leading to size-assortative mating in amphibians. With data from natural populations collected over several years, we first show a consistent pattern of size-assortative mating across our 2 study populations. We subsequently ask if assortative mating may be explained by mate availability due to temporal segregation of migrating individuals with specific sizes. With additional experiments, we finally assess whether size-assortative mating is adaptive, i.e. influenced by mating competition among males, or by reduced fertilization in size-mismatched pairs. We find that size-assortative mating is in accordance with differences in mate availability during migration, where larger individuals of both sexes reach breeding ponds earlier than smaller individuals. We observe an indiscriminate mate choice behavior of small males and an advantage of larger males pairing with females during scramble competition. The tactic of small males, to be faster and less discriminative than large males, may increase their chances to get access to females. Experimental tests indicate that the fertilization success is not affected by size assortment. However, since female fecundity is highly correlated with body size, males preferring larger females should maximize their number of offspring. Therefore, we conclude that in this frog species mate choice is more complex than formerly believed.

Key words: **amphibia, evolution, male–male competition, reproductive strategy, assortment by chance.**

INTRODUCTION

Reproductive success is the most important aspect of individual fitness. Consequently, various mating systems, strategies, and tactics have evolved, and they may vary between and within species (Gross 1996; Shuster and Wade 2003). Random mating would mean that all individuals of a given population would mate with the same probabilities, but due to natural and sexual selection, physical constraints, and stratification of populations, nonrandom mating is the rule in taxa with sexual reproduction (Crespi 1989; Otronen 1993; Arnqvist et al. 1996; Harari et al. 1999; Bearhop et al. 2005; Taborsky et al. 2009). One common pattern of nonrandom mating is assortative mating, defined by the correlation of traits (phenotypic or genotypic) across mated pairs. Although the strength of assortment differs between taxa and traits, the direction of assortment is usually positive, i.e. individuals with similar traits are more likely to mate (Thiessen and Gregg 1980; Crespi 1989; Acord et al. 2013; Jiang et al. 2013). Negative assortment occurs if offspring may have advantages from trait dissimilarity of their parents, e.g. assortment to maximize diversity of major histocompatibility complex alleles (Meyer and Thomson 2001; Mays and Hill 2004), or advantages of heterozygotes (Hedrick et al. 2016). Assortment can also be incidental, due to spatial or temporal segregation (Jiang

Address correspondence to M.-O. Rödel. E-mail: mo.roedel@mfn-berlin.de.

et al. 2013). Examples for such incidents causing assortative mating include spatial and/or temporal separation in birds (Bearhop et al. 2005), temporal segregation of *Drosophila* strains (Tauber et al. 2003), or differences in flowering periods in plants (Devaux and Lande 2008; Weis et al. 2014).

In anuran amphibians (frogs and toads), size-assortative mating is frequently observed, but the underlying causes have rarely been elucidated (Arak 1983; Halliday 1983; Howard and Kluge 1985; Sullivan et al. 1995). Mostly, size assortment is associated with male mate choice; when males compete directly over females, the access to females is limited, and the fertility of females is size dependent (Krupa 1995). A limited access to females leads to high variation in male mating success (Jones et al. 2002). Therefore, competition among males for females is common and considerably high in explosive or lek-breeding species (Wells 1977; Arak 1983; Bradbury and Gibson 1983). This competition can be expressed as direct combat between males, dominance of specific males, territoriality, or other tactics—e.g. satellite males—to gain access to females (Wells 1977; Shine 1979; Arak 1983; Tsuji and Matsui 2002). These mating tactics are often not fixed and the behavior of a nonpaired individual is status and context dependent and may thus change over its lifetime (Dominey 1984; Lucas et al. 1996; Bowcock et al. 2013). Fertility of anuran females is usually positively correlated with female body size (Wells 2007; Nali et al. 2014) and, therefore, males should prefer to mate with larger females to increase their reproductive fitness.

The first and most obvious scenario leading to size-assortative mating relies on competitive advantages of large males securing mating with the preferred large females (Berven 1981; Howard and Kluge 1985), e.g. due to their stronger grip in amplexus and better combat performance. Thus, pairs of large individuals are formed while small "left-over" females would mate with similarly small males. A second mechanism that could lead to assortative mating derives from the fact that reproductive success does not merely depend on the total number of eggs produced by a female but rather on the number of "fertilized" eggs sired by a male. In various explosive breeding anurans multiple paternity has been observed (Laurila and Seppä 1998; Lodé and Lesbarrères 2004; Vieites et al. 2004), which can occur through other males fertilizing those eggs that were left unfertilized by the amplecting male. This suggests that a substantial proportion of eggs are not immediately fertilized by the amplecting male. In particular, the distance between female to male cloaca may influence fertilization success, and thus fitness of mates in species with external fertilization such as most anurans (Davies and Halliday 1977; Robertson 1990). A third proximate factor that could lead to size assortment is the temporal sorting of differently sized individuals, where individuals of similar size arrive at similar times at the breeding sites (Howard and Kluge 1985; Ryser 1989; Elmberg 1990; Lodé et al. 2005). This could be due to physiological reasons (Morbey and Ydenberg 2001), e.g. larger individuals can store more energy reserves, have higher migration abilities, are less prone to desiccation, and could therefore start migration earlier under less favorable weather conditions (Elmberg 1991; Kovar et al. 2009). Furthermore, individuals hibernate in different overwintering sites and distances to ponds vary (Pasanen and Sorjonen 1994).

The European common frog, *Rana temporaria* Linnaeus, 1758, is a widespread Palearctic species and occurs in a variety of different habitats. Common frogs are explosive breeders; individuals aggregate in large numbers at the breeding sites for approximately 2 weeks in early spring (Gollmann et al. 2014). Usually, the operational sex ratio (OSR) at the breeding site is male-biased (Elmberg 1990; Vojar et al. 2015), which leads to male–male competition. Males show different mating tactics that seem to be size and frequency dependent. Small males can be seen searching/waiting for females at the edge of the breeding pond, while larger males seem to aggregate within the breeding pond, participating in scramble competition (Arak 1983). These larger males are more often successful in female takeover attempts than the smaller ones (Savage 1961). Therefore, it should be beneficial and cost effective for smaller males to be less picky in choosing a mate, also known as the concept of prudent choice (Härdling and Kokko 2005). If they are faster in grabbing a female, the chance to keep a female until spawning is increasing. We therefore hypothesize that, based on a combination of male mate choice, male–male competition and an evolutionary advantage of maximized fertilization success by size-matched pairs, sexual selection in *R. temporaria* might result in size assortment of mates. Additionally, incidental assortment due to migration patterns could favor assortment. Here, we use field data from 2 *R. temporaria* populations and experiments, to examine the mechanisms leading to pair-formation in populations of *R. temporaria*. We differentiated between mechanisms leading to size assortment during the migration period to the breeding pond where male densities are low and therefore mate choice could play a more prominently role; and during scramble competition within the pond where male densities and competition are high. We hypothesize that:

1) Smaller males should be faster in grabbing a female, if larger males have an advantage in male–male competition. As male–male competition is supposedly stronger within ponds, pairs caught within ponds should therefore show stronger size assortment than pairs caught outside ponds.
2) Larger individuals arrive first at the breeding sites, and size matching of pairs is partly due to temporal migration patterns.
3) If the relative distance between cloacae affects the fertilization success of pairs during amplexus, we expect size-matched pairs to show a greater fertilization success.

MATERIAL AND METHODS

Study areas

The study was carried out at 2 areas in southern and central Germany. The first is located in the deciduous beech forest surrounding the village Fabrikschleichach, Lower Franconia (49.924 N, 10.555 E; hereafter FS). This area contains a network of 140 ponds, where *R. temporaria* annually uses between 35 and 40 ponds for reproduction. In 2010, and in 2013 to 2016, we fenced 3–6 ponds, which have been continuously used for reproduction since 2005 (Grözinger et al. 2012), in order to catch pairs and single individuals outside the ponds. The fence consisted of plastic gauze (mesh size 2 mm, height approximately 60 cm) stretched between wooden poles. The ponds remained fenced for the entire reproductive period (1–2 weeks; 2010: 17–31 March; 2013: 02–17 April; 2014: 15–21 March; 2015: 14–31 March; 2016: 15 March–01 April). We installed buckets buried to the ground level along the exterior fence side (every 5 m), to collect arriving individuals. Fence and buckets were controlled twice a day (morning and evening), and all individuals (nonpaired males, $n = 714$; nonpaired females, $n = 193$) and pairs ($n = 597$) found were sexed and measured in situ. We measured snout-vent-length (SVL) using a caliper (in mm, to the closest 0.5 mm), and mass using a spring scale (1–100 g, 1 g increments). Additionally, the date and mating status (nonpaired or in amplexus) of arriving individuals was noted.

The second field site was located near Braunschweig, Lower Saxony, Germany. Here, fieldwork was carried out at the locality Kleiwiesen (52.328 N, 10.582 E; hereafter KW), which comprises a system of ponds surrounded by meadows and mixed deciduous beech forest, sustaining a large population of *R. temporaria*. According to our observations over a period of 10 years, almost the complete population breeds in a small shallow part of one pond, partly covered with dense reeds. Field observations were primarily carried out at night and began when the first pair was found and ended when there were no more pairs found (10–26 March 2012 and 08–16 April 2013). We caught all pairs (n = 174), nonpaired males (n = 412) and nonpaired females (n = 8) by hand from within the ponds and measured them on site for SVL and weight. Individuals were released only after completing measuring procedures to avoid recaptures.

Size-assortative mating in the field

We tested if size-related mating patterns in *R. temporaria* are nonrandom and measured snout-vent-length SVL of nonpaired and paired individuals in different years and locations. Size data (SVL) of pairs were tested for their relationship with a Pearson correlation and the respective 95% confidence interval was calculated. In FS we found pairs of *R. temporaria* along the fence and within buckets. The latter theoretically could lead to biased results, i.e. larger males replacing smaller, already amplectant ones, especially in buckets where several pairs were trapped simultaneously. Therefore, we conducted separate analyses for pairs in and outside of buckets. For all statistical analyses, we used R software (Version 3.4.0., R Core Team 2017). The package ggplot2 was used for visualization (Wickham 2009). The mean SVL of paired versus nonpaired males and females was compared in each population with a Welsh 2 sample t-test and Cohen's d was calculated as standardized effect size (R package effsize; Torchiano 2017). If differences were present, this would be a sign for nonrandom mating patterns, mate choice behavior and/or male–male competition. Furthermore, we calculated the intensity of sexual selection (ISS), defined as the standardized difference between the mean size of paired males and the mean size of all males in the population (Arnold and Wade 1984). This metric presents the shift of the mean value, caused by selection, in units of standard deviations for the specific phenotypic trait (Arnold and Wade 1984). The values of the male–female size ratio, defined as the SVL of the male divided by the SVL of the female, were compared with a Welsh 2 sample t-test to examine if size matching differed between locations. We compared the size matching ratios of our natural populations to the values we achieved with artificial pairing during the fertilization success experiment, to make sure the latter represent ratios found in nature.

Temporal migration pattern

Temporal or spatial migration patterns of differently sized animals can lead to incidental assortment at the breeding site (Bearhop et al. 2005; Jiang et al. 2013). It is known from some explosive breeding anurans that larger males arrive first at the breeding site (Howard and Kluge 1985; Elmberg 1990). For the FS population, we collected data on day of appearance at the fence and tested if body size was decreasing with migration time, which could lead to an incidental size assortment during migration (total individuals: n = 2098). We fitted a linear mixed model (LMM) on body size with day of appearance and sex as fixed factors and year as random factor. To fit the model we used the lmer function in the R package lme4 (Bates et al. 2015) with restricted maximum likelihood and calculated the marginal and conditional coefficients of determination (R^2) with the R package MuMIn (Bartoń 2016). The influence of fixed effects was tested with a Wald Chi^2 test and that of the random effect with a restricted likelihood ratio test (RLRsim package; Scheipl et al. 2008).

Mating speed experiment

To test if male body size has an effect on time until mating in *R. temporaria*, we carried out a mating speed experiment in KW. Differently sized males in breeding condition; 1 large male (64–79 mm SVL) and 1 small male (54–64 mm SVL); were confronted with gravid females (58–68 mm SVL, in-between the SVL values of the 2 males, Figure 5). We then recorded occurrence of amplexus and the size of the successful male. This experiment was short-term, amplexus typically occurring within minutes and rarely after periods >1 h. Spawning did not occur in any of the trials. This experiment aimed to see which male (small vs. large) is faster in grabbing a medium sized female. The 3 test subjects were placed together in a water-filled container (diameter ca. 30 cm; water depth ca. 15 cm), and as soon as 1 male was observed in amplexus with a female it was recorded whether it was the smaller or the larger male. Each specimen (88 males, 44 females) was used for a single trial only (n = 44). The data were analyzed with a binomial test, where "small male grabs the female first" was defined as success. Additionally, we calculated a logistic regression model with binomial distributed response variable (success = small male first, failure = large male first) to find the variables that explain the observed pattern best. R^2 was calculated with the MuMIn package (Bartoń 2016). We used the Zelig package (Choirat et al. 2016) to simulate the probability of the small male winning in dependency of the large males' body size by using the logistic regression model. Therefore, we set mean size of females and mean size of small males as fixed variables and run the simulation over the range of the large males SVL with 1000 simulations.

Fertilization success experiment

This experiment was designed to test whether assortative mating might confer a direct selective advantage by avoiding low fertilization rates that are known to occur with large size differences in anuran pairs (Davies and Halliday 1977; Robertson 1990). Therefore, we collected amplectant pairs in the KW area between 21 March 2015 and 1 May, 2015 (n = 45). Pairs were disengaged and transferred to the laboratory in buckets filled with water. In the lab, new pairs were placed separately in plastic tanks (dimensions 40 cm length, 22 cm width, 13 cm height) with 5–10 cm water and kept in a ventilated basement experiencing natural daily fluctuations in air pressure and temperature (tank water temperature mean ± SD = 12.0 ± 1.8°C, range = 6.5–14°C; measured by a iButton® Thermochron at 1 h intervals). The SVL of males and females was measured with a caliper to the nearest 1 mm, and pairs were arranged to achieve a broad range of size ratio values. We counted the total number of eggs produced within 48 h after spawning by placing the clutch in a light yellow plastic container to assure a high contrast between box and eggs. Eggs were carefully distributed across the bottom of the containers with little water and later slightly flattened with the aid of a transparent acrylic sheet. We took photographs and processed them with the spot detection function in Icy software (de Chaumont et al. 2012). After 7 days, another picture was taken and the number of undeveloped eggs was counted on screen to guarantee a precise discrimination of eggs and early larvae (Gosner stages 17–20, Gosner 1960). The task of automatically recognizing and counting the larvae in the 7 days clutch pictures was complicated, as they move and adopt many different shapes hence, instead of counting the larvae we counted

the number of remaining (undeveloped) eggs, a much simpler image recognition task, and used the initial egg count as a reference for the calculations. We removed one completely unfertilized clutch from the dataset (total $n = 44$). The fertilization success was defined by the ratio of developed larvae to the number of deposited eggs (expressed as percent). For statistical analysis, we used a logistic regression model with binomial distribution of the response variable (success = number of embryos, fail = number of unfertilized eggs) and size-ratio of pairs as explanatory variable. A second approach was looking at male SVL as explanatory variable for fertilization success (logistic regression with binomial distribution). The SVL of male anurans could influence the fertilization success because bigger males produce a higher number of spermatozoa (Smith-Gill and Berven 1980; Edwards et al. 2004).

RESULTS

Size-assortative mating in the field

We detected positive size-assortative mating in both locations in almost all years, except in FS 2015 and KW 2013. The Pearson correlation coefficient (r) and the corresponding 95% confidence interval (CI) per year are given in Figure 1. The degree of assortment differed between pairs which were formed within buckets (pair in) and those that formed outside of buckets (pair out) but none of the groups showed consistently higher levels of assortment. In general, the CI increased with decreasing sample size and OSR had no influence on degree of assortment (Table 1). Detailed values per day can be found in the supplementary material (Supplementary Table S1).

The SVL of amplectant males did not differ from that of nonpaired males in FS, where pairs were intercepted while migrating to the ponds (Welch 2 sample t-test, $t = -1.39$, $P = 0.1661$, $d = -0.08$) and in most years we observed only negligible effect sizes, i.e. variance of body size between the groups is not different from the variance within the group (Table 1). The intensity of sexual selection (ISS) was mostly small and negative in FS, showing that paired males were slightly smaller than nonpaired males (Figure 2, Table 1). Paired females in FS were significantly larger than nonpaired ones (Welsh 2 sample t-test, $t = 4.20$, $P < 0.001$, $d = 0.35$) and effect sizes were always

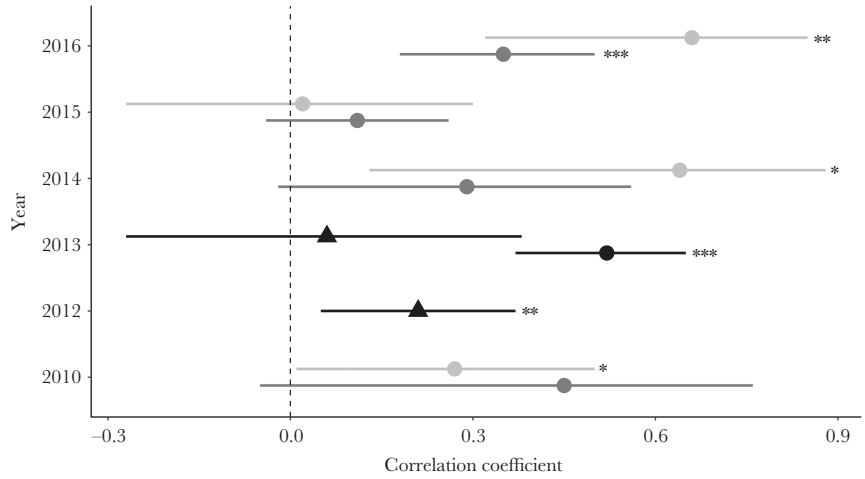

Figure 1
Correlation coefficient with respective 95% confidence interval of size assortment (snout-vent-length) of amplectant pairs of *R. temporaria* in the localities Fabrikschleichach (circle) and Kleiwiesen (triangle). Correlation coefficients are given for each year and are separated by pairs (black), pairs found inside of buckets (dark gray) and outside buckets at the fence (light gray). The black dotted line represents zero correlation. Significant correlations are marked with * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Table 1
Summary of body sizes of *R. temporaria* pairs (Males and Females) found in 2 populations over several years, with effect size Cohen´s d and intensity of sexual selection

year	site	status	n	OSR	SVL M	d M	d CI 95%	ISS M	SVL F	d F	d CI 95%	ISS F
2010	FS	pair_in	17	1.2	69.18	0.64	0.03–1.24	0.87	71.14	0.34	−0.35–1.02	0.04
		pair_out	55		67.25	0.15	−0.28–0.75	−0.04	71.54	0.28	−0.25–0.81	0.19
2013		pair	107	1.5	70.8	−0.12	−0.37–0.13	−0.18	74.74	0.51	0.16–0.85	0.44
2014		pair_in	39	1.3	70.65	0.01	−0.42–0.42	0.11	75.51	0.29	−0.19–0.77	0.3
		pair_out	13		68.85	−0.34	−0.96–0.28	−0.76	75.46	0.3	−0.37–0.97	0.28
2015		pair_in	176	2.1	69.96	−0.05	−0.23–0.14	−0.07	74.45	0.32	−0.1–0.74	0.17
		pair_out	48		70.08	−0.03	−0.33–0.28	−0.02	73.27	0.15	−0.34–0.64	−0.29
2016		pair_in	121	1.5	64.17	−0.13	−0.36–0.11	−0.21	67.41	0.09	−0.21–0.39	0.1
		pair_out	21		65.43	0.08	−0.38–0.53	0.74	66.76	0.01	−0.5–0.49	−0.15
2012	KW	pair	137	2.5	71.19	0.48	0.26–0.69	0.68	66.73	0.49	−0.23–1.21	0.07
2013		pair	37	3.3	73.96	0.53	0.13–0.93	0.81	71.65	NA	NA	NA

Sites: FS = Fabrikschleichach, KW = Kleiwiesen; Status: pair in = inside of buckets, pair out = outside of buckets; OSR: operational sex ration (n males/n females per year); d = Cohen's d; d CI 95%: corresponding 95% confidence interval of d; ISS: intensity of sexual selection.

positive with small to medium effect, i.e. the variance of body size between groups is higher than within the group and can be explained by the "effect" pairing. The ISS was mostly positive (Table 1).

In KW, where pairs and single individuals were sampled in the breeding pond, and thus with potentially higher levels of scramble competition than in FS, the amplectant males were significantly larger than nonpaired ones (Welch 2 sample *t*-test, $t = 4.38$, $P < 0.001$, $d = 0.43$, Figure 2). When comparing paired males with all males within a population, the ISS was higher in KW than in FS (Table 1). Paired females were larger in KW (Figure 2). However, this difference was not significant (Welsh 2 sample *t*-test, $t = 1.51$, $P = 0.1724$, $d = 0.63$), probably due to small sample size of nonpaired females ($n = 8$). The male to female size ratio (measured as male SVL divided by female SVL) differed significantly between the locations (Welsh 2 sample *t*-test, $t = -10.54$, $P < 0.001$, $d = -0.99$). In FS, the mean pair size ratio was less than 1, (mean ± SD; 0.95 ± 0.10), i.e. females were larger than males (mean ± SD; SVL males: 68.6 ± 6.2 mm; SVL females: 72.4 ± 7.5 mm); although males were larger than females in KW (mean ± SD; 1.06 ± 0.12; SVL males: 71.2 ± 6.0 mm; SVL females: 67.8 ± 6.5 mm) (Figure 3).

Temporal migration pattern

At fenced ponds in FS, we found a consistent pattern of large specimens arriving earlier at the pond for both sexes, at almost all years (Figure 4). The LMM showed that body size was decreasing with ongoing time of migration within the year (Wald Chi^2 test, $\chi^2 = 108.26$, df = 1, $P < 0.001$) for both sexes and that males were smaller than females (Wald Chi^2 test, $\chi^2 = 136.88$, df = 1, $P < 0.001$). The fixed effects day and sex explained 13% of variance in our model (marginal R^2). The year had an influence on frogs' sizes (restricted likelihood ratio test, RLRT = 287.77, $P < 0.001$); when including year as random effect, the model explained 25% of variation in size differences (conditional R^2, Supplementary Table S2).

Mating speed experiment

Small males were 36% more successful (faster) in grabbing females than respective larger males. From 44 trials, smaller ones won in 30 of them ($n = 44$, 2-tailed Binomial test, $P = 0.02$, Figure 5). The logistic regression model for this experiment ($n = 44$) suggested that large male SVL has an influence on the winning probability of the small male ($Z = 2.1$, $P < 0.05$, Supplementary Table S3). In our simulation, we could see that the probability of the small male grabbing the female first increases as the SVL of the relatively larger male in an experiment increased (Figure 6). This indicates that the larger males got slower. Additionally, the size difference between the relatively larger male and the female seems to play a role. We observed a smaller size difference of large male to female when the large male grabbed her first (mean ± SD; 6.7 ± 3.9 mm) compared to when the small male grabbed her first (mean ± SD; 8.4 ± 4.5 mm). However, this difference was not significant (Welsh *t*-test, $t = 1.26$, $P = 0.219$, $d = 0.39$).

Fertilization success experiment

A total of 44 pairs mated and deposited eggs successfully in the laboratory. The male/female SVL ratio of breeders ranged between 0.78 and 1.30 (mean ± SD; 1.02 ± 0.13). The number of deposited eggs per female (mean ± SD, range; 1259 ± 384, 653–2213 eggs) was positively correlated with female size (mean ± SD, range; 65.5 ± 5.6, 55.3–80.3 mm; Pearson $r = 0.90$, 95% CI = 0.83–0.95, $P < 0.001$), where female size is accounting for approximately 80% of the variation in number of deposited eggs (linear regression, $R^2 = 0.81$, $F_{(1,42)} = 168.2$, $P < 0.001$). The average fertilization success was relatively high but showed a wide range (mean ± SD, range; 85.6 ± 18.6%, 16.9–99.2% fertilization success, Figure 7). The logistic regression analyses showed no influence of size-ratio values or male SVL on fertilization success. The fit of these models was very poor and explained almost none of the variation in the dataset. Therefore, with our experimental approach, we could not detect an influence of size ratio on fertilization success.

DISCUSSION

Indications for a complex, multicausal size-assortative mating pattern

As expected from other studies (Berven 1981; Arak 1983; Gibbons and McCarthy 1986; Vojar et al. 2015), we detected positive size-assortative mating in both *R. temporaria* populations, and we

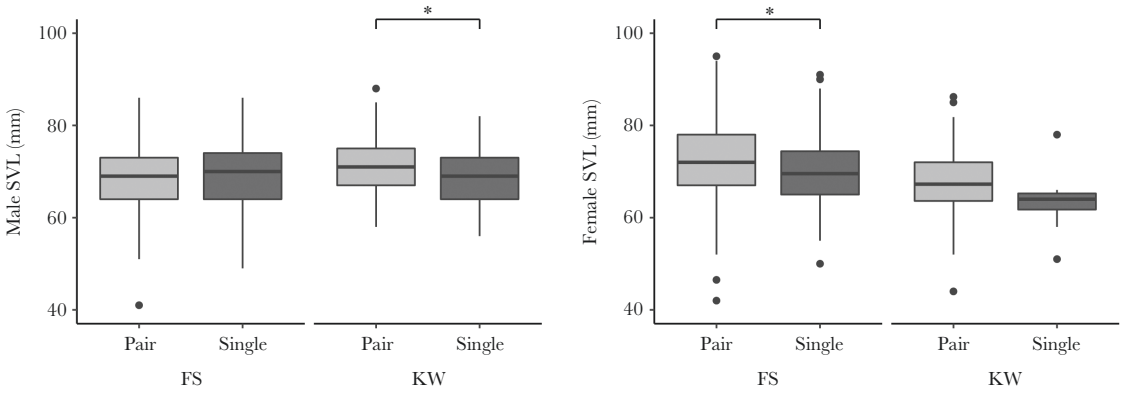

Figure 2
Differences of SVL between paired and nonpaired *R. temporaria* males (left) and females (right) at both study sites. Although no size differences were detected in Fabrikschleichach (FS) between paired ($n = 597$) and nonpaired males ($n = 709$), in Kleiwiesen (KW) paired males ($n = 174$) were significantly larger than nonpaired males ($n = 285$; $P < 0.001$, $d = 0.43$). Paired females ($n = 597$) in FS were significantly larger than nonpaired females ($n = 193$; $P < 0.001$, $d = 0.35$). In KW size difference was not significant; paired females ($n = 174$) did not differ from nonpaired ones ($n = 8$; $P = 0.17$, $d = 0.63$). The boxplots show median (dark line), 25–75% quartile (box), nonoutlier range (vertical line) and outliers (black dots). An asterisk depicts significant differences (*$P < 0.05$).

observed body size differences between paired and nonpaired individuals. Such a size-assortative pattern is typically interpreted as a consequence of male mate choice. We observed a migration pattern, where larger individuals arrive first at the breeding pond, which supports size assortment by temporal covariation. However, our experiment indicated that mating tactics differ between small and large-sized males. The small males appear to be faster in grabbing a female than larger males. This pattern suggests that the explanation of size-assortative mating in these frogs is not as straightforward as it might seem at the first glance. In the following, we discuss the evidence for 3 main factors that might influence size-assortative mating in this species: temporal migration pattern, competitive male–male displacement and different mating tactics, and increased fertilization success of size-matched pairs.

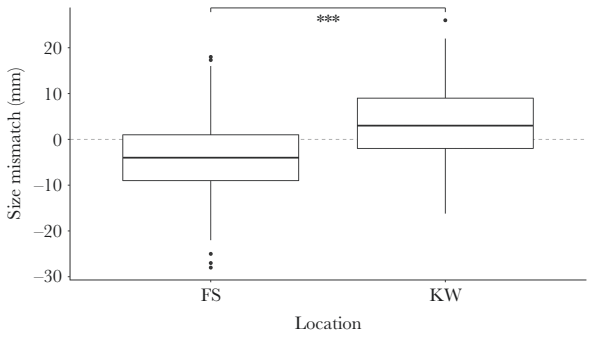

Figure 3
Size ratio of amplectant pairs of *R. temporaria* in Fabrikschleichach (males were smaller than females, $n = 597$) and Kleiwiesen (males were bigger than females, $n = 174$). The boxplots show median (dark line), 25–75% quartile (box), nonoutlier range (vertical line) and outliers (black dots). The dashed line depicts equal size of male and female. Between locations the difference of size ratio in pairs was significant and is depicted by 3 asterisks (Welsh 2 sample *t*-test, $t = -10.54$, $P < 0.001$, $d = -0.99$).

Size assortment during migration

In FS, we fenced the pond before migration started, and single frogs and pairs were intercepted at the fence. The pairs forming terrestrially, outside of the breeding aggregation, had to face a lower male density, resulting in a lower operational sex ratio, and therefore less competition between males (Höglund 1989; Byrne and Roberts 2004). Paired and nonpaired FS males did not differ in body size with a tendency of smaller males being paired, which supports the theory of less competition and the absence of large male advantage. The chances for smaller males to gain access to a large high quality female should be higher at low male densities (Arak 1988). Therefore, we expected a lower strength of size assortment. Still, we observed a positive size assortment of pairs, which could be

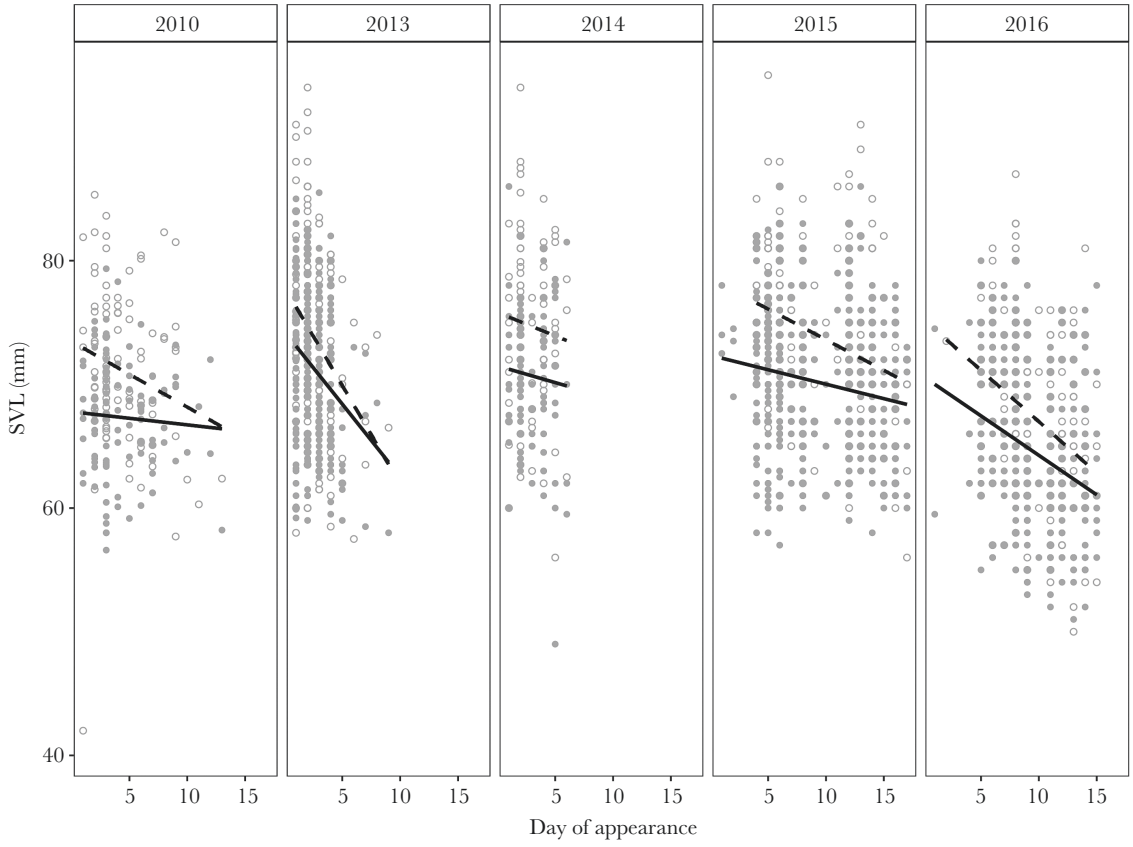

Figure 4
Relationship of appearance day at the fence (FS) and SVL in *R. temporaria* (open circles = females, filled circles = males). The plots show individual data per year and the respective linear regression line (dashed line = females, solid line = males).

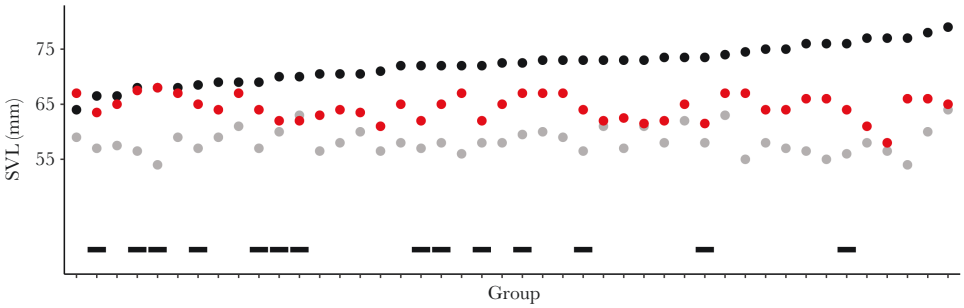

Figure 5
Mate speed experiment. Small males (gray), large males (black), and females (red) per trial. Black bars indicate the large male grabbing the female first. (n = 44). The groups are sorted by increasing large male SVL.

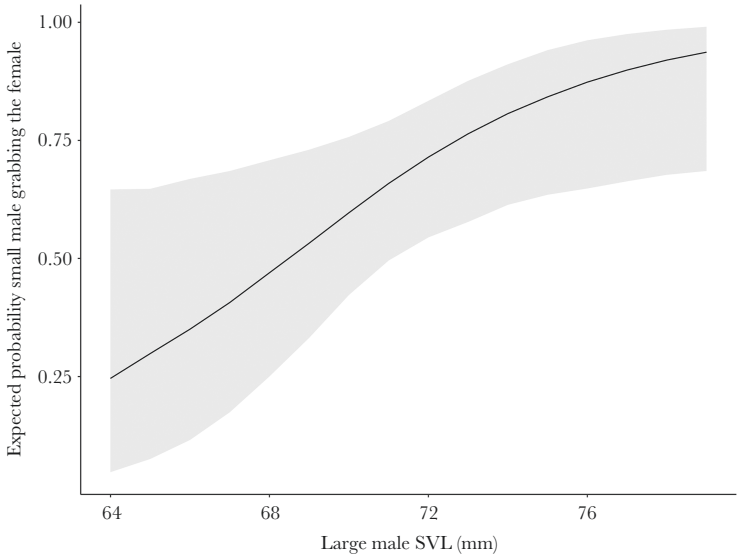

Figure 6
Probability of the small male grabbing the female first. Simulation was done with average female SVL (64.4 mm), average small male SVL (58.2 mm) and the range of large male SVL (64–79 mm). Given is the median probability of the small male grabbing the female first (black) and the 95% confidence interval (gray). The probability of the small male grabbing the female first is increasing with the increase of the large male's body size.

due to temporal covariation that we observed at the fence, where larger individuals (males and females) arrived first at the breeding site. So far, this has been reported for males (Howard and Kluge 1985; Loman and Madsen 1986; Elmberg 1990) and rarely for females (Lodé et al. 2005), but the reasons behind this arrival pattern are not fully clarified (Wells 2007). The pattern could be due to physiological reasons, e.g. higher energy constrains of small individuals (Ryser 1989) or desiccation risk (Thorson 1955), which limits migration time and distances. Also, simple mechanistic reasons could lead to an earlier arrival of large individuals, since they are faster and can cover larger distances (Zug 1978). However, migration distances are highly variable (Pasanen and Sorjonen 1994; Kovar et al. 2009). Former experience of finding breeding ponds by larger, and thus older, individuals knowing the available sites better could be an important factor (Reading 2001). In addition, timing of arrival at the breeding site can influence survival of adults and eggs/larvae, either through unfavorable weather conditions like freezing or heat waves (Pasanen and Karhapää 1997; Håkansson and Loman 2004), or through predation (Heusser 1970; Lodé et al. 2004). Larger animals are less prone to freezing or desiccation, due to their surface/volume ratio; and they are too big for some predators. Furthermore, reproductive success can be influenced by arrival time, e.g. multiple mating in males can cause depleted sperm storages, energy reserves, or decreased mating motivation (Smith 1976; Gibbons and McCarthy 1986; Elmberg 1991; Hettyey et al. 2009a), which could therefore affect females that arrive late in the breeding season. Despite temporal covariation, size assortment during migration could be due to different mating tactics shown by small and large males, which are mostly density dependent (Arak 1988; Lodé et al. 2004). It can be expected that all males show a preference for large females to maximize their reproductive output, and that this preference is highest when there is less competition and therefore less costs (Fawcett and Johnstone 2003). This hypothesis is supported by our observation of paired females being larger than nonpaired females. Arriving early and grabbing a (high quality) female could have energetic benefits for small and large males, because the rate of male replacement is considered to be very low in most anurans (<5%, Wells 2007). Pairs of *R. temporaria* have been seen in amplexus for several hours or days without spawning (Geisselmann et al. 1971; Elmberg 1990; and own

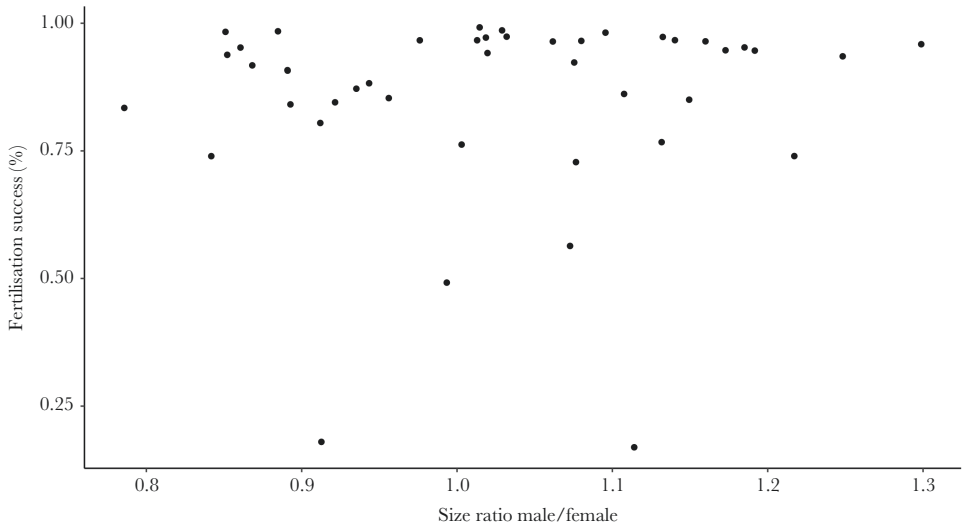

Figure 7
Relationship between size ratio (male/female SVL) and fertilization success in pairs of *R. temporaria* (n = 44). We could not detect any relationship between size ratio of pairs and fertilization success.

observations), which is considered to be a strategy of mate guarding (Savage 1961; Wells 1977; Arak 1983). However, the strategy of a prolonged amplexus could likewise favor female mate choice (Krupa 1995). It was shown that females are able to retain eggs if in amplexus with an unfavorable male and to prolong the prespawning period (Reyer et al. 1999; Hettyey et al. 2009b). Extended egg retainment (e.g. over several days) would provide females the possibility to test the endurance of the amplecting male, eventually leading to displacement of less perseverant males by others (Hettyey et al. 2009b). A *R. temporaria* male tactic to reduce the period during which they are exposed to possible male–male displacement fights might be to induce spawning by the application of pheromone proteins through the skin-abrasions on the female belly generated during amplexus (Willaert et al. 2013). However, more research is needed to test whether small males might produce these amplexin peptides more readily or in higher quantities to counteract potential female choice.

We believe that the size assortment in FS is primarily a result of temporal covariation during migration, subsequently modulated by mate choice behavior and low male–male competition.

Size assortment during scramble competition

It has been previously shown that higher male densities in *R. temporaria* lead to a stronger size assortment (Vojar et al. 2015) and large-male advantage (Arak 1983; Elmberg 1991). In KW, the pairs were caught within the pond breeding aggregation where male densities are higher than in the terrestrial environment. Here, we observed paired males to be larger than nonpaired males, which could be a consequence of large-male advantage during scramble competition (Wells 1977; Höglund 1989; Byrne and Roberts 2004). For larger males it could pay off to fight for a large female, in order to maximize the potential number of eggs to fertilize, because large females have higher fecundity (our results; Howard and Kluge 1985; Ryser 1989; Elmberg 1991; Lardner and Loman 2003). Costs for maintaining a high quality female could thus be comparatively high for small males, as losing such a female to a larger male during scramble competition seems likely. Thus, the most successful tactics available for small males should be a prudent choice of smaller females (Härdling and Kokko 2005); or the unselective tactic of immediately grabbing any female. We saw in our mating speed experiments that on average, smaller males were faster in grabbing a female, which could be a consequence of unselective behavior of small males (Wells 1977), of the prudent choice of at least some small-sized males (Härdling and Kokko 2005), or simply of small males being more agile and swift in grabbing a female than their larger competitors. However, it is also possible that the females in the experiments were too small to trigger an amplexus behavior in the larger male (Kroupa 1995), as they were always—at least a bit—smaller than the larger male.

To conclude, male–male displacement in *R. temporaria* certainly occurs, but male–male displacement fights are not the immediate consequence of most encounters of single males with pairs, probably also due to the fact that single males might employ different mating tactics, like prudent or indiscriminate mate choice.

Size-assortative mating and fertilization success

A positive size assortment could also arise from an active choice of similarly sized mates. Such a behavior may be adaptive if fertilization of eggs was compromised in size-mismatched pairs. In anurans, the influence of male/female size ratios on fertilization success is highly variable (Wogel et al. 2005). Experimental evidence in *R. temporaria* is mixed. Gibbons and McCarthy (1986) found a positive influence on the fertilization success when males have been larger, whereas Elmberg (1991) found the fertilization success being all or none and independent from male/female size ratio. Likewise, we could not detect a relationship between the size ratio of pairs and the percentage of fertilized eggs. In our experiment, the size ratio of pairs was comparable to the ones found in our natural populations and in former experimental studies (Gibbons and McCarthy 1986), and fertilization rates were comparable with former studies in nature (Gibbons and McCarthy 1986; Vieites et al. 2004). However, fertilization success is influenced by many different factors, which might confound such experimental results. This includes temperature, acidity (Beattie 1980; Freda 1986),

number of former mating by the male (Gibbons and McCarthy 1986; Hettyey et al. 2009a) or the timely synchronization of gamete output/ejection, as well as sperm quality, sperm competition and genetic compatibility (Dziminski et al. 2009; Sherman et al. 2010; Álvarez et al. 2014).

Also, multiple paternities are a common phenomenon in lek-breeding anurans (Roberts et al. 1999; Lodé and Lesbarrères 2004; Merilä and Knopp 2009). In *R. temporaria*, multiple paternities can be caused by "clutch piracy", where a satellite male grasps a clutch and fertilizes the eggs in the center of the clutch (Vieites et al. 2004; see Supplementary S4 for an example video of a breeding aggregation in FS, where a couple of nonpaired males enter a freshly laid clutch), but theoretically might also be caused by "stray sperm" (Laurila and Seppä 1998). In our experiment, stray sperm could have increased fertilization success in the limited amount of water, however, similar fertilization rates have been observed in nature (Gibbons and McCarthy 1986; Vieites et al. 2004).

CONCLUSION

The complex mating system of *R. temporaria* is embedded in a multicausal framework. We believe that the temporal migration pattern plays an important role in the formation of size assortment during the migration period. If large males arrive earlier at the pond and gather in the shallow parts where spawning takes place, it would be beneficial for later arriving small males to stay at the pond edges and wait for the arriving females. As shown before, the large males have an advantage in scramble competition, are probably more successful in takeover attempts and could better hold on to a female. But, if a small male can grab a female before she is entering the breeding aggregation, the chances to stay with her until spawning occurs are good. Under higher levels of male competition, prudent or indiscriminate mate choice could be a successful mating tactic for smaller males. Therefore, size assortment is modulated by temporal covariation, male–male competition, male mate choice behavior, and seems to have no effect on fertilization success.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at https://academic.oup.com/beheco/

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Conflict of Interest: We have no competing interest.

Ethical Statement: Research and handling permissions, according to German nature protection laws, were given for fieldwork in Lower Franconia by Regierung Unterfranken. Fieldwork in Lower Saxony was carried out with permits of Stadt Braunschweig and Landkreis Helmstedt, and experiments approved by the ethics committee of Lower Saxony.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Dittrich et al. (2017).

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Paper 2

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Correspondence

Description of female release calls of the European Common Frog, *Rana temporaria* (Anura: Ranidae)

Carolin Dittrich[1] & Mark-Oliver Rödel[1,2]

[1)] Museum für Naturkunde Berlin, Leibniz Institute for Evolution and Biodiversity Science, Invalidenstraße 43, 10115 Berlin, Germany
[2)] Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

Corresponding author: Carolin Dittrich, e-mail: carolin.dittrich@mfn.berlin

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Anuran vocalization research has been conducted since the mid twentieth century, when calls were described and categorized according to their social context (Toledo et al. 2015). As a result, calls connected to mating and courtship are well studied, as they permit species recognition and are thus used for studies covering taxonomy, behavioural ecology, and monitoring programs (Wells 1977, Dorcas et al. 2009, Köhler et al. 2017).

The European Common Frog, *Rana temporaria* Linnaeus, 1758, is an explosive breeder with dense breeding aggregations in spring that are mainly male biased (Savage 1961). The advertisement call is not primarily addressed to the females, but to keep the chorus of males' clustered (Savage 1961, Van Gelder et al. 1978). Another function of the advertisement call could be the maintenance of the reproductive status in males, by keeping the androgen hormone levels high (Brzoska & Obert 1980).

The possibility of female mate choice in explosive breeding anurans is usually neglected (Wells 1977). Indeed, most publications state that female mate choice is precluded by scramble competition between males (Green et al. 2019) and those females are passive during the process of reproduction (Gollmann et al. 2014). Already in 1758, Rosenhof said that "then I have noticed that the female sometimes grunts too, but not so often and loudly"[translated from German]. Savage (1934) described Common Frog females producing grunting noises after deposition of their eggs, to signal their non-receptivity towards males. This publication lacked the description of the call, however. A release call of non-receptive females was described later by Brzoska et al. (1977) and is characterized by two frequency bands at 1100–1300 Hz and 1700–2000 Hz, respectively, and 18 pulses per call.

In this study, we describe two different release calls for *R. temporaria* females, compare them to the previously mentioned publications and discuss their possible behavioural context.

In spring 2019, we conducted behavioural experiments to investigate mate choice behaviour in *R. temporaria*. We performed fieldwork in the surroundings of the ecological field station of the University of Würzburg, in Fabrikschleichach (49.924 N, 10.555 E). We recorded experimental mate choice behaviour for one hour per experiment with a web camera with two internal microphones (Logitech C920) connected to a MacBook Pro. The webcam was attached to a tripod at 1.5 m height. The webcam settings permitted for a sampling rate of 44.1 kHz (247 kBit/s) and are recorded in a compressed mov-format. No filters or noise reduction have been used during recording; therefore, the recordings have a poor quality.

For the mate choice experiments, we put one male and two differently sized females in a plastic container (40 × 60 × 40 cm), filled with 10 l of rainwater (5 cm high). The containers were standing in the barn of the field station and air temperatures ranged from 5 to 15°C.

We converted those video sequences where males or females were calling into an audio wav-file (sampling rate: 44.1 kHz, 16-bit) and compared the calls to published spectrograms (Brzoska et al. 1977, Van Gelder et al. 1978). We down-sampled the wav-files in CoolEdit (sampling rate 11050 Hz, mono, 16-bit) and we used a bandpassfilter of 200–2000 Hz for the calls to remove background noise. If necessary, we removed background artefacts up to 300 Hz. We analysed the calls with Avisoft Bioacoustic software (Avisoft SASLab Pro Version 5.2.13, R. Specht, Glienicke, Germany). The configuration for the analyses was the following; FFT length: 1024, win-

Table 1. Properties of *Rana temporaria* female calls. Given are the female ID, which pair they belonged to, the females' snout–vent length in mm, the call type, average duration per single call in ms and the respective standard deviation, number of calls in a call series, average inter-call interval duration in ms, average minimum and maximum frequency from a call series and average dominant frequency of the call series (all in Hz).

ID	pair	SVL (mm)	call type	duration (ms)	n calls	interval (ms)	minimum frequency (Hz)	maximum frequency (Hz)	dominant frequency (Hz)
315	2	69	squeak	0.16 ± 0.01	5	1.21 ± 0.38	993 ± 69	1100 ± 74	1032 ± 92
349	5	65	squeak	0.15 ± 0.01	4	1.06 ± 0.38	824 ± 13	983 ± 36	953 ± 38
34	39	68	squeak	0.14 ± 0.02	10	0.73 ± 0.18	1105 ± 106	1285 ± 108	1222 ± 130
18	15	74	squeak	0.17 ± 0.03	8	2.84 ± 1.18	536 ± 196	1076 ± 37	985 ± 26
72	33	54	squeak	0.16 ± 0.04	6	2.23 ± 1.69	836 ± 42	1103 ± 206	951 ± 128
59	35	78	squeak	0.11 ± 0.04	5	1.55 ± 0.63	625 ± 125	977 ± 199	762 ± 36
8	18	53	squeak	0.15 ± 0.02	6	0.85 ± 0.51	1129 ± 41	1281 ± 26	1210 ± 52
35	31	71	squeak	0.15 ± 0.05	5	1.36 ± 0.88	859 ± 6	967 ± 49	884 ± 9
79	42	69	squeak	0.14 ± 0.02	5	1.19 ± 1.21	873 ± 272	1080 ± 106	954 ± 248
27	17	58	squeak	0.20 ± 0.02	11	0.95 ± 0.37	668 ± 116	1071 ± 33	914 ± 53
28	17	75	squeak	0.20 ± 0.02	4	0.97 ± 0.06	669 ± 24	1096 ± 73	884 ± 72
56	30	63	squeak	0.12 ± 0.02	17	0.52 ± 0.07	440 ± 187	1054 ± 82	826 ± 141
7	16	68	squeak	0.18 ± 0.03	12	1.27 ± 0.48	443 ± 53	1289 ± 68	802 ± 221
70	41	77	squeak	0.24 ± 0.04	13	0.73 ± 0.26	439 ± 48	1105 ± 229	699 ± 50
110	52	72	squeak	0.15 ± 0.03	9	2.32 ± 2.50	529 ± 91	946 ± 88	767 ± 101
92	46	65	grunt	0.17 ± 0.03	8	0.84 ± 0.23	435 ± 55	1124 ± 253	499 ± 27
107	49	74	grunt	0.20 ± 0.03	15	0.60 ± 0.11	237 ± 16	934 ± 90	295 ± 54
66	44	75	grunt	0.13 ± 0.03	4	2.28 ± 2.10	446 ± 6	958 ± 21	502 ± 69
58	34	75	grunt	0.18 ± 0.02	22	0.67 ± 0.14	364 ± 26	1180 ± 124	528 ± 134
12	23	63	grunt	0.16 ± 0.03	7	1.32 ± 0.88	330 ± 57	995 ± 161	588 ± 128
114	50	69	grunt	0.15 ± 0.02	12	0.88 ± 0.27	292 ± 20	889 ± 86	401 ± 153
26	13	86	grunt	0.21 ± 0.04	32	1.73 ± 1.29	263 ± 33	877 ± 28	403 ± 52

dow type: Bartlett, bandwidth: 56 Hz, resolution: 43 Hz, overlap: 93.75%, temporal resolution: 1.4512 ms. We measured the duration of the single calls, the number of calls in a call series, the inter-call interval, the minimum and maximum frequency and the dominant frequency per call. The spectrograms were drawn with R statistical software (R Core Team 2019, R version 3.6.1) and the packages seewave 2.1.4 (Sueur et al. 2008) and tuneR 1.3.3 (Ligges et al. 2018). We provide the parameters for the drawing of the spectrograms at the respective figures. The terminology and analysis of the call description follows the recommendations by Köhler et al. (2017). We analysed calls of two males (number of single calls, n = 12) and release calls from 22 females (number of single calls, n = 220). All sound files are deposited at the animal sound archive (https://www.tierstimmenarchiv.de/webinterface/contents/searchtext.php) of the Museum für Naturkunde, Berlin (archive numbers: Rana_temporaria_DIG0204_01–DIG0204_23).

The male calls fitted the known pattern and structure of the *R. temporaria* advertisement call, which is described having a frequency band between 300–900 Hz with a maximum frequency between 350–500 Hz, and two higher frequency bands at 1000–1400 Hz and 1400–1900 Hz (Brzoska et al. 1977). In our experiment 16 out of 41 males in amplexus called. This was in particular observed, when the female was moving and trying to free herself from amplexus. The male calls showed a dominant frequency of 521 ± 103 Hz (n males = 2, n single calls = 12, bandwidth: 296–1890 Hz, Fig. 1).

In our experiments, 26 females evoked calls and we were able to use 22 female calls for bioacoustics analyses (Table 1). Release calls are defined as audible calls emitted while tentatively amplected or touched by a male and are mostly coupled with little body side vibrations of the female (Toledo et al. 2015). We identified two distinct female release calls when females were amplected by a male, which were emitted directly after the male touched the female and whilst vibrations were observed on the flanks of the female. These release calls differed in their frequency distribution, but were similar in structure: a single unpulsed, non-frequency modulated simple call and a short duration of single calls in a call series. We defined all calls with an average dominant frequency below 600 Hz as a grunting sound and all calls with a dominant frequency above 600 Hz as a squeaking sound.

The first call type was a grunting sound with a dominant frequency of 459 ± 91 Hz (n females = 7, n single calls = 100), a minimum frequency of 338 ± 75 Hz and maximum frequency of 994 ± 108 Hz. The average duration of one single call was 0.17 ± 0.03 ms and average inter-call interval was 1.19 ± 0.62 ms. Females released 4–32 of these calls in

a series. We provide the spectrogram and oscillogram of a grunting sound in Figure 2.

The second call type was a squeaking sound with a dominant frequency of 923 ± 146 Hz (n females = 15, n single calls = 120), a minimum frequency of 731 ± 227 Hz and maximum frequency of 1094 ± 108 Hz. The average duration of one single call was 0.16 ± 0.03 ms and average intercall interval was 1.32 ± 0.66 ms. The females released 4–17 of these calls in a series. We provide the spectrogram and oscillogram of a squeaking sound in Figure 3.

The body size of an individual influences the frequency, that is larger animals produce lower frequencies (Wells 2007). We observed a significant negative correlation of snout–vent length and average dominant frequency (Fig. 4; Pearson correlation, n = 22, r = -0.49, p = 0.02). Individuals of similar body size were observed emitting both sounds, which indicates two different call types that are not simply depending on body size.

The calls of the males examined in our study corresponded in form and structure to the advertisement call described be Brzoska et al. (1977) and the B-call described by Van Gelder et al. (1978). In our experiments, these calls were emitted when the males grabbed a female and she was trying to free herself from amplexus. Savage (1934) stated that the male calling should keep the chorus clustered and is not addressed solely to the females. However, in our examples the call seems to be addressed to the struggling female only, and could be described as an amplectant call (Toledo et al. 2015). Therefore, we hypothesize that this call might also have a calming or comforting function towards the female.

The females emitted two different sound types that functionally seemed to be both release calls, but differed in their dominant frequencies. The squeaking sound with higher dominant frequencies seems to be the one described by Brzoska et al. (1977). The female grunting sound with lower dominant frequencies was more similar to the male release calls that show dominant frequencies around 200–300 Hz (Brzoska et al. 1977) and therefore, might have a different function than the squeaking release call. We as-

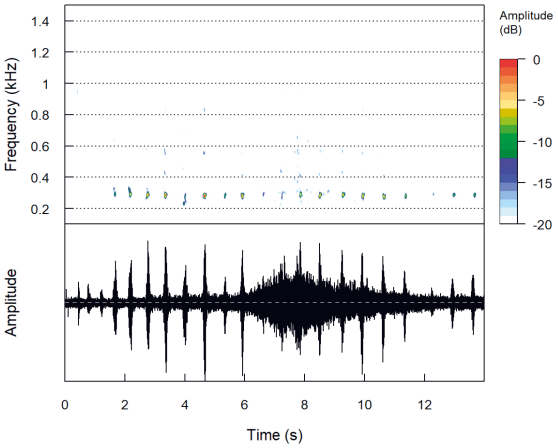

Figure 2. Spectrogram and oscillogram of *Rana temporaria* female ID107 (SVL: 74 mm; pair 49; TSA: Rana_temporaria_DIG0204_21) emitting grunting sound after being grabbed by a male. Dominant frequency; average ± SD: 295 ± 54 Hz; bandwidth: 237–934 Hz. Frequencies at second 6–10 are background noise. Spectrogram parameters: sampling frequency 11025 Hz, window length 1024, Hamming window.

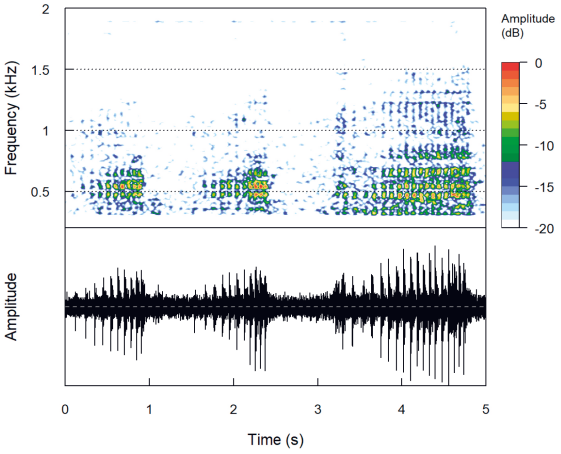

Figure 1. Spectrogram and oscillogram of a *Rana temporaria* male advertisement call (SVL: 62 mm; pair 44; Tierstimmenarchiv (TSA): Rana_temporaria_DIG0204_02). Dominant frequency; average ± SD: 492 ± 34 Hz; bandwidth: 293–1890 Hz. Spectrogram parameters: sampling frequency 11025 Hz, window length 1024, Hamming window, cut out second 9 to 14.

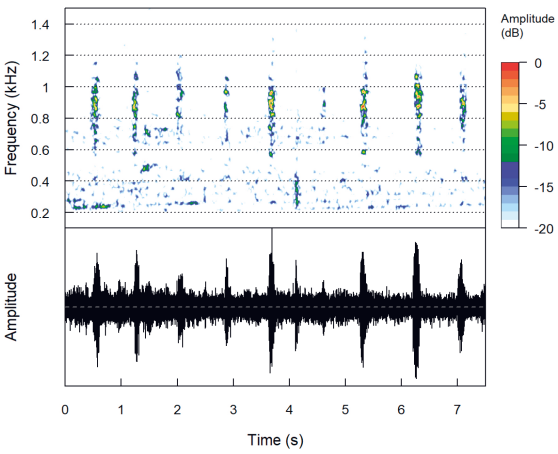

Figure 3. Spectrogram and oscillogram of *Rana temporaria* female ID27 (SVL: 58 mm; pair 17; TSA: Rana_temporaria_DIG0204_07) emitting squeaking sound after being grabbed by a male. Dominant frequency; average ± SD: 914 ± 53 Hz; bandwidth: 550–1148 Hz. Spectrogram parameters: sampling frequency 11025 Hz, window length 1024, Hamming window, cut seconds 3.5 to 11.

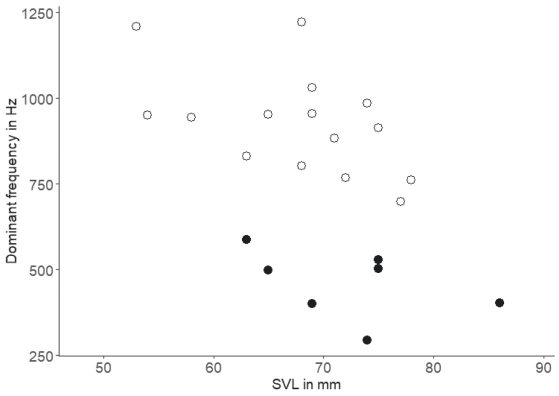

Figure 4. Snout–vent length in mm per *Rana temporaria* female and their respective average dominant frequency of the emitted female call in Hz. Dark dots represent the grunting sound, light dots the squeaking sound (compare text).

sume that the grunting release call might imitate the males' release call, and thus lead to a potentially faster release of females from amplexus, without spawning taking place. This hypothesis will be tested elsewhere. In support of our theory, the A-call described by Van Gelder et al. (1978) has similar dominant frequencies and lead more often to the release of other frogs than other call types. In addition, Savage (1961) described the male release call (he termed it "warning-croak") as a grunting sound and states that this sound is emitted by females as a signal for their un-readiness to mate (Savage 1934).

Herein, we have shown that female *Rana temporaria* may emit two different release calls when grabbed by a male, whereas Brzoska et al. (1977) had already described one with a higher dominant frequency, above 600 Hz. The second call, mentioned by Savage (1934), seems to imitate the male release call and may lead to a higher rate of successful escapes by females from amplexus.

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Paper 3

The freedom of choice – Female mate choice behaviour in *Rana temporaria*.

Dittrich, C., & Rödel, M.-O. (manuscript in prep.) The freedom of choice – Female mate choice behaviour in *Rana temporaria*.

The freedom of choice - Female mate choice in *Rana temporaria*

Carolin Dittrich[1] & Mark-Oliver Rödel[1,2]

The theory of sexual selection states that in most species females are the choosy sex, in which choosiness is the individual effort to invest energy and time to assess potential mates. In most amphibians, female mate choice is the rule rather than the exception. They choose their mating partners depending on call probabilities, quality of territory or genetic compatibility. Only in explosive breeding anurans, high intrasexual competition should preclude female choice and favour males to be the choosy sex. In the current study, we observed pair formation in the European Common Frog without intrasexual competition. We wanted to know, if males are choosy considering female body size and more importantly, if females really cannot choose their mating partner. We conducted mate choice experiments placing a male and two differently sized females in a box and recorded their mating behaviour. We found that mate choice by males was random. There was no preference for larger over smaller females (or vice versa). Instead, females are the ones to choose their mating partner, in accordance with sexual selection theory. Females can apply different mate choice behaviours to increase their chances to escape from a male. Either they could rotate their bodies to free themselves, emit release calls or they feign death. We witnessed a high failure rate of males, attempting to grab a female, which would make male mate choice a costly trait. Herein we show that females are the ones who have the freedom of choice, despite being an explosive breeding species.

Sexual selection and mate choice have been a controversially discussed topic during the 19th century when Darwin (1871) published his book, "The descent of man, and selection in relation to sex". His fellow scholars easily accepted the theory of male combat, but the idea of females actively taking part in sexual selection remained neglected until the mid-twentieth century. During the last decades, the research on sexual selection, female/male mate choice and mating systems increased considerably (Janetos, 1980; Ryan and Keddy-Hector, 1992; Paul, 2002; Edward and Chapman, 2011). Looking at the theory of sexual selection females are the choosy sex in most species, where choosiness is defined as the individual effort to invest energy and time to mate assessment (Jennions and Petrie, 1997). They invest more energy in the production of eggs than males do in the production of sperm (Trivers, 1972). Therefore, females should choose a male to mate with, to provide their offspring with the best possible genes to increase survival chances and therefore her personal reproductive fitness (Møller and Alatalo, 1999). Besides that indirect benefit of mate choice, females could also gain direct benefits from resources provided by the male or parental care (Kirkpatrick and Ryan, 1991; Kokko and Jennions, 2008). Mating systems in amphibians are diverse and should depend on female availability over time (Wells, 2007). In anuran species, frogs and toads, which have a long breeding time (prolonged breeders), female mate choice seems to be the rule rather than an exception (Wells, 1977). Females actively choose a male by its calling abilities (Toledo et al., 2015; Ryan et al., 2019) or the quality of his defended territory and availability of resources (Howard, 1978; da Rocha et al., 2018). In contrast, in explosive breeding anuran species that have a short breeding time, males engage in scramble competition and are considered the choosy sex (Wells, 1977; Berven, 1981), with little possibilities for females to choose (Howard, 1980; Halliday, 1983; Green, 2019). Mate choice in males should occur when female fecundity is size dependent (Krupa, 1995). In many anuran amphibians, this is the case (Nali et al., 2014) and therefore, males should favour the largest females to mate with to increase their reproductive fitness. This argumentation focuses on intrasexual competition only and lacks the possibility of female mate choice, although both mechanism should shape mating patterns and therefore sexual selection (Darwin, 1871). The European Common Frog (*Rana temporaria* Linneaus, 1758) is an explosive breeding species that forms large breeding aggregations in early spring (Gollmann et al., 2014). The mating behaviour and breeding of this species is well studied (Haapanen, 1982; Ryser, 1989), at least compared to other anuran species. Males engage in scramble competition (Savage, 1961), were larger males have an advantage in taking over receptive females (Arak, 1983). Males do not show any preferences in mate choice considering female body size, body mass or fecundity (Elmberg, 1991) and mating is random (Elmberg, 1987; Ryser, 1989), although intraspecific variability in mating patterns are reported and non-random mating can be found as well (Arak, 1983; Dittrich et al., 2018). None of the mentioned studies took the possibility of female mate choice into account. However, there is evidence from older literature that females of *R. temporaria* do show mate choice behaviour. They gutter the same grunting noises as the males, which became known as male release calls (Rosenhof, 1758). They would do so after they deposited eggs or to signal non-receptiveness towards a male (Savage, 1934). A description of these release call was submitted for publication recently (paper 2), together with another call, formerly described by Brzoska et al. (1977). Furthermore, Savage (1934) added that a female was feigning death when clasped by a male, but until now, no experimental data on mate choice behaviour in females of the Common frog are available. Regardless of these older publications, the recent literature states that females are passive during courtship and reproductive behaviour (Gollmann et al., 2014). In this study, we investigate the mating behaviour of the European Common Frog because we question the passivity of females described in the recent literature. Therefore, we will focus on the following two hypotheses:

1. In the absence of intrasexuel competition males will show a preference to mate with large females.
2. Females are not passive and will display mate choice behaviour.

[1]Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity, D-10115 Berlin, Germany [2] Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), D-10115 Berlin, Germany

Material and Methods

Study area and species

We carried out fieldwork in southern Germany, near the village Fabrikschleichach in Lower Franconia, Bavaria (49.924 N, 10.555 E). This area comprises about 140 ponds, of which *Rana temporaria* uses between 35 – 40 ponds for reproduction annually. We fenced the four ponds with the largest known breeding aggregations for the entire reproductive period from 14th to 28th of March 2019. The fence consisted of plastic gauze (mesh size 2 mm, height approx. 60 cm) stretched between wooden poles and was controlled twice a day (morning and evening). We collected individuals that sat at the fence or were on their way to the breeding pond, preferably collecting singles to minimize differences in reproductive status. Amplectant females could potentially be affected by the application of amplexin (Willaert et al., 2013). So far, it is unknown if male *R. temporaria* are able to detect differences in the females reproductive status (Thomas, 2011). We thus always took note if individuals were encountered as singles or paired. All individuals were sexed in–situ. We measured snout–vent length (SVL) using a calliper (in mm, to the closest 0.5 mm), and mass using a spring scale (1 – 100 g, 1 g increments). For transport, we placed each individual in a 1 L volume plastic bucket, which was equipped with leaf litter to hide and a thin layer of water to prevent desiccation. The animals were kept in these buckets in the barn of the ecological field station in Fabrikschleichach (temperatures only marginally higher than at the breeding sites) until the start of the behavioural experiments.

Behavioural experiments

In the behavioural mate choice test, we aimed testing the hypothesis that all males prefer the largest female in the absence of intrasexual competition. Therefore, we placed two females of different body size categories (small and large) in one container, in which size difference between females in a trial had to exceed 10 mm. Overall the small female was smaller than 70 mm (n = 48; 48 – 70 mm), the large female larger than 71 mm (n = 48, 71 – 89 mm). We tested 23 small males (56 – 70 mm) and 25 large males (71 – 89 mm). The experiments were conducted in plastic containers (40 x 60 x 40 cm), filled with 10 L of rainwater (5 cm high). Before starting the experiment, a non-transparent plastic sheet separated the male from both females. We let the animals acclimatise in the container for 10 min, then removed the plastic sheet and started the experiment. We attached a web camera (Logitech C920) at 1.5 m height and recorded the experiment for one hour. Before starting a new experiment, we cleaned the containers and exchanged the water completely to minimize the risk of potential contamination by any kind of chemical cues. Each animal was tested only once. If amplexus did not occur after a maximum of one hour, the trial was terminated. In the night following an experiment, all respective animals were returned to the pond of origin. After screening the videos we defined several variables which were recorded: when and towards which female the male attempted first, the number of successful and failed clasping attempts on each female, and female mate choice behaviour; i.e. rotating, release call, and death feigning. Here, we first define the different behaviours. Attempt of a male: a male moves towards a female and tries to grab her, an attempt can be successful and the male gets hold of the female or it can fail and the female escapes (Video S1). Female behaviour: a) rotating: when grabbed by a male the female is trying to rotate her body while the male tries to counter-balance her movement (Fig. 1; Video S2) b) release call: when grabbed by the male the female starts calling; there are two distinct calls emitted, a grunting and a squeaking sound (Audio S3 and S4) c) death feigning: female feigns death when grabbed by a male with female's arms and legs far stretched (Fig. 2; Video S5)

Figure 1 – *Rana temporaria* pair, the female (darker, lower frog) tries to rotate herself out of amplexus and the male is counterbalancing the movement with his right foot (lighter, upper frog)

Figure 2 – *Rana temporaria* female (right) feigning death until the male (left) let's her go (drawings from video sequences: Sebastian Arsand).

Statistics

We used a binomial test to analyse if the first attempt or the choice of the male was random or significantly different from random. The rates of female mate choice behaviours shown by small and large females were compared with a Chi square test. We tested with a t-test, if the size difference

within pairs (female minus male SVL) influenced female mate choice behaviour, and calculated Cohen´s D as effect size. The potential influence of size differences within pairs on escape probabilities of females from amplexus, was modelled with a binomial logit model. We compared the escape rates of small and large females by applying a two-sided Kolmogorov-Smirnov test. All analysis and graphs were done in the R statistical environment version 3.6.1 (R Core Team, 2018). We used the packages effsize (Torchiano, 2019), ggplot2 (Wickham, 2009), gridExtra (Auguie, 2017), MuMIn (Barton, 2019), plyr (Wickham, 2011) and Zelig (Imai et al., 2008; Choirat et al., 2018).

Results

Male mate choice

In total, we conducted 48 experiments (23 with a small male, 25 with a large male). From these 48 trials, 32 ended in the formation of pairs; 16 experiments were terminated after one hour without an amplexus formed. Small females have been in amplexus in 13 trials, large females in 19 trials (binomial test, $p = 0.38$; Fig. 3a). The males did not show any preference considering female body size and attempted to grab small and large females almost equally often during their first attempt (small female n = 24, large female n = 21; binomial test, $p = 0.77$; Fig. 3b). In total, males attempted to grab a female 509 times and failed in 356 cases (70%).

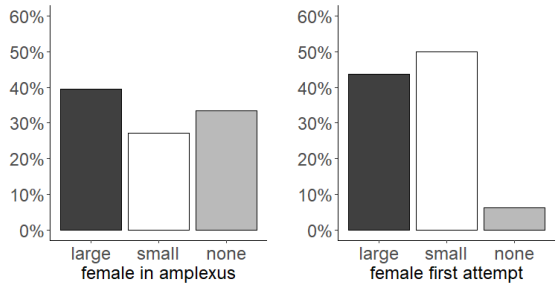

Figure 3 – Percentage of *Rana temporaria* males being in amplexus with a large (dark bar) or small (white bar) female (left). In one third of all experiments (n = 16) no amplexus occurred (grey bar); males directing their first attempt towards large (dark) or small (light) females (right); only 6.25% of the males did not show any interest for a female (grey). No preferences were shown, neither in amplexus nor in first attempt of males towards large or small females.

Female mate choice

During our experiments, females displayed three behaviours of mate choice that lead to a release from amplexus with a male; 1) rotating, 2) release calls, and 3) feigning death. In total, 54 females were in amplexus with a male. One or more mate choice behaviours were shown by 47 of those females (small females n = 27, large females n = 20). Males clasped and stayed with the female if she was not showing any kind of the three behaviours (n = 7). First, we tested the influence of former amplexus on being amplexed again and could not detect a difference between the status as a single (n = 75) or amplexed female (n = 21) (Chi square test, $\chi^2 = 1.10$, df = 1, $p = 0.30$). Second, we tested the influence of former amplexus on the display of mate choice behaviour and it did not differ (Chi square test, $\chi^2 = 1.74$, df = 1, $p = 0.19$). Therefore, we pooled all data. The most common behaviour of mate choice was rotating with 83% of all females in amplexus showing this behaviour (Fig. 4). Small females (48%) did show this behaviour more often than large females (35%). However, this difference was not significant (Chi square test, $\chi^2 = 0.54$, df = 1, $p = 0.46$). Release calls were emitted by 48% of amplected females. Thereby small females emitted the squeaking sound more often than the grunting sound (Fig. 4), although this difference was not significant (Chi square test, $\chi^2 = 0.57$, df = 1, $p = 0.45$). Female calls were always associated with rotating. Death feigning was observed in 33% of all amplected females. Small females showed this behaviour in 24% and large females in only 9% of the trials (Fig. 4; Chi square test, $\chi^2 = 3.04$, df = 1, $p = 0.08$).

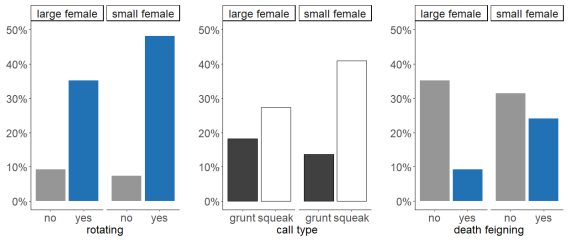

Figure 4 – Percentage of *Rana temporaria* females showing rotating behaviour to free themselves from amplexus with a male (blue bars)(left). Small females show that behaviour in 48% of all trials, large females in 35% of all trials; this difference was not significant. Female release call types (middle, for further details see paper 2). Small females released more often the squeaking sound, but this difference was not significant. Females feigning death while in amplexus (right). Small females show that behaviour more often (blue bars); however, this difference was not significant.

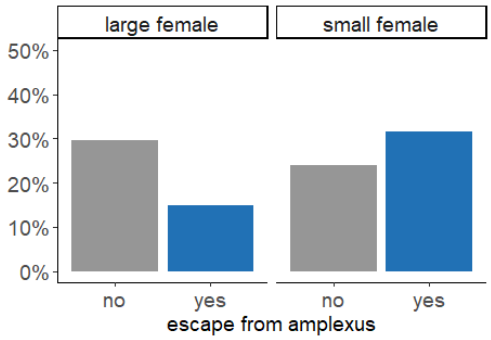

Figure 5 – Female *Rana temporaria* escape rates from amplexus. In total 46% of amplected females could free themselves from the male (blue bars). Small females (31%) escaped more often than large females (15%).

Overall, the application of mate choice behaviour let to the release of 25 females, which had been in amplexus (46%). Small females escaped amplexus more than twice

as often as large females (small females n = 17, large females n = 8, Fig. 5). However, this differences was not statistically significant (Chi square test, $\chi^2 = 2.9197$, df = 1, $p = 0.09$).

In general, small females show mate choice behaviours a little more often than large females, but the differences were not statistically significant. Additionally, small females escaped from a male more often than larger females. We calculated escape rates for each mate choice behaviour and separated them by size class of females. Escape rates were slightly higher, although not significant, for smaller females when showing one of the behaviours (Fig. 6; two-sample Kolmogorov-Smirnov test, D = 0.6, $p = 0.33$).

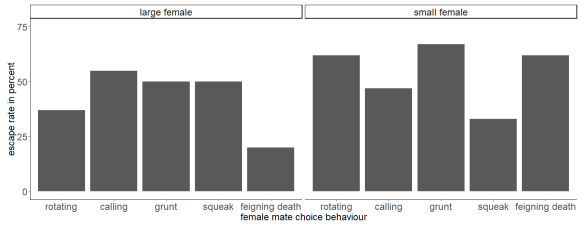

Figure 6 – Escape rates in percent by *Rana temporaria* females when showing female mate choice behaviour to escape from amplexus, separated by female size category.

We observed that small males attempted to grab small females more often (small females n = 12, large females n = 9), but were in amplexus with larger females more often (small n = 5, large n = 10). Therefore, we tested if the size difference within pairs had any effect on the display of mate choice behaviour and on a successful escape (Table 1). When rotating was applied, females had been on average smaller than the males, although not significant (Table 1). The females that applied rotating and escaped were on average smaller than the males, though not significant (Fig. 7a). However, when looking at the effect size Cohen´s D, we observed a medium effect size comparing the females that showed rotating and escaped successfully, to the females that did not escape. This implies that size difference between female and male could have an effect on escape chances (Table 1). Nevertheless, by applying one of the behaviours escape rates have been on average 50%.

Size difference in pairs did not have an effect on calling behaviour in females (Table 1). The females that called and escaped were on average slightly larger than the males, but not significantly (Fig. 7b). We observed a small effect size comparing the size difference of pairs in which females where calling and escaped successfully to the pairs in which females called and did not escape (Table 1). In paper 2 we described the two different sounds emitted by the female, a grunting and a squeaking sound. We assume that the grunting release call is imitating the male release call, which could lead to higher escape rates of females. In support of this theory, five of eight females that emitted grunting sounds successfully escaped their respective male, but only six out of 15 squeaking sounds lead to a successful escape of the female (Fig. 7c). When death feigning was applied females were on average smaller than males, although not significant (Table 1). We observed a small effect size comparing the size difference of pairs in which

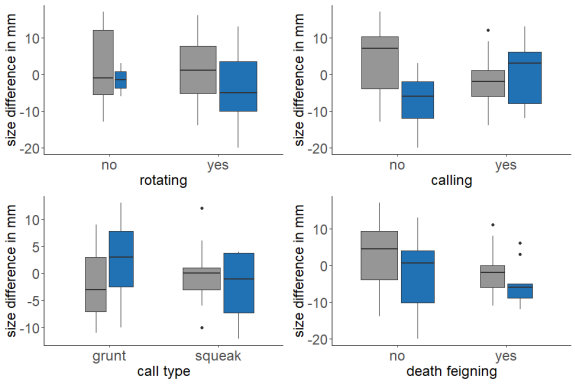

Figure 7 – *Rana temporaria* female mate choice behaviour and the respective size difference between female and male (negative value when females are smaller than the male, positive value when the female is larger than the male). The grey boxplots show pairs in which the females did not escape, the blue boxplot the pairs in which females did escape: The width of the boxplot varies with number of observations (larger width represents higher n); a) rotating, b) calling (all female calls), c) call type and d) death feigning.

females feigned death and the ones that did not (Table 1). The females that feigned death and escaped, were on average smaller than the males, though not significant (Fig. 7d). However, we observed a medium effect size comparing the size difference of pairs in which the females feigned death and escaped successfully to the females that feigned death and did not escape (Table 1).

Table 1 – Comparing body size (SVL) difference between male and female *Rana temporaria* pairs when applying mate choice behaviour (MCB). In the first test we compare size differences in pairs when behaviour was shown by a female *versus* behaviour was not shown. In the second test we compare size differences in pairs when behaviour was shown by a female and lead to successful escape *versus* no escape; statistical tests (t-test) and effect sizes (Cohen´s D) are given.

MCB	behaviour yes – no				behaviour yes; escape yes – no			
	yes	no	t-test	D	yes	no	t-test	D
rotating	-1.27 ± 8.81	1.44 ± 10.33	t= 0.74 p= 0.48	0.29	-3.52 ± 8.76	1.09 ± 8.42	t= 1.8 p= 0.08	0.54
calling	-2.08 ± 10.36	-0.4 ± 10.62	t= -0.10 p= 0.92	0.03	0.15 ± 8.14	-1.54 ± 7.68	t= -0.55 p= 0.59	0.21
feigning death	-3.33 ± 6.76	0.44 ± 9.83	t= 1.65 p= 0.11	0.42	-5.11 ± 5.97	-1.56 ± 7.37	t= 1.13 p= 0.28	0.53

It thus seems that size difference in pairs might have an influence on escape probabilities. Therefore, we calculated a binomial logit model to test the influence of size difference on escape probabilities. The model showed an effect of size difference, in which escape probability increased when the female was smaller than the male (Fig. 8), though not significant ($p = 0.06$) with small explained variance (likelihood-ratio adjusted $R^2 = 0.09$).

Discussion

The freedom of choice with whom to reproduce in the European Common Frog seems to be a female based one. Contrary to our expectations, males do not prefer certain females – although they should, but females seemed to do so

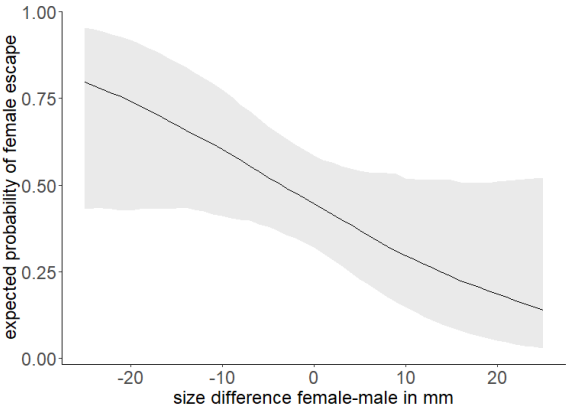

Figure 8 – Binomial model predicting the escape probability of a female *Rana temporaria* in amplexus, depending on the size difference of pairs (negative value when females are smaller than the male, positive value when the female is larger than the male). Given are the median value (black line) and the 95% confidence interval (grey).

– contrary to the literature. We showed that *Rana temporaria* males were not performing size based mate choice. We revealed that *R. temporaria* females are displaying active mate choice, and thus preference behaviour, shown by body rotation, release calls and feigning death, towards a male. The chances to free themselves from the male by applying one of the behaviours lay around 50% and chances were not increasing when applying several behaviours together.

One general assumption on the evolution of mate choice is an interplay between male secondary sexual traits and the preference of these traits by conspecific females (Kirkpatrick, 1985). Furthermore, choosiness is costly regarding energy and time spend to choose a mate (Jennions and Petrie, 1997). Time is a limiting factor in explosive breeding species and time constraints could be an important factor in the evolution of mate choice strategies (Sullivan, 1994). Acceptance or rejection of a mate depends upon amplexus time, which can vary in *R. temporaria* from a couple of hours (Savage, 1934) to days in the "pre-spawn season" (Savage, 1935). Males are usually the first to arrive and stay longer at the breeding pond (Savage, 1961; Geisselmann et al., 1971; Berven, 1981). If males spend too much time to search and to get access to a female, energy reserves after hibernation could decrease dramatically, as weight loss is a linear function of days spend at the pond, which applies to both sexes (Ryser, 1989). However, male mating success depends on their attendance and persistence at the breeding site (Woodward, 1982). This energy loss could decrease the competitive abilities during scramble competition and weaken the strength to hold a female until spawning occurs. Therefore, chances to reproduce in the given year are decreasing over time, especially when most females in the breeding aggregation are paired already. Females will certainly find a mating partner if she made it successfully to the pond, therefore she could take more time to sample several males to choose from, depending on her arrival time to the breeding aggregation, although sampling should be costly (Jennions and Petrie, 1997). Males on the other hand should keep sampling time to a minimum to ensure reproduction in the recent season, as time of breeding is limited and the operational sex ratio male-biased (Elmberg, 1990; Dittrich et al., 2018).

Not only intrasexual competition, but also the high failure rate in attempting a female should favour non-choosiness in males. In our study, 70% of all attempts to clasp a female failed. This high rate of failure may be one reason that male mate choice should not be a trait under selection. Additionally, not only that males should invest energy and time to search for a mate, they are also prone to a higher risk of predation (Lodé et al., 2004; Igaune et al., 2008), due to the conspicuous behaviour of males searching for females or calling during the reproductive season (Arak, 1983; Ryan, 1985). If predation risk increases, the effort to discriminate or sample between mates decreases (Jennions and Petrie, 1997). On the long run, the survival of the male should be more important for the male lifetime mating success than competition (Elmberg, 1990). In an evolutionary context, it could be too costly for males to be choosy (Krupa, 1995; Fawcett and Johnstone, 2003).

Body size alone should not be a trait under sexual selection in anuran amphibians, because it is age and resource dependent (Green, 2019), even though larger males do have a mating advantage in a variety of anuran species (Woodward, 1982; Höglund, 1989; Tejedo, 1992; Böll and Linsenmair, 1998). Indeed, female and male mate choice seems to be independent from body size and therefore from age and survival probabilities. However, mate choice should be based on multiple cues, e.g. body size, colouration, calling abilities, chemical communication or genetic incompatibility (Engeler and Reyer, 2001; Taylor et al., 2007; Willaert et al., 2013; Starnberger et al., 2014; Bossuyt et al., 2019), which act together. Another important component is detectability of these cues, which could differ based on age and /or body size (Ronald et al., 2012). Female body size could influence mate choice, due to body size related perception of stimuli (Jennions et al., 1995). In general, we observed mate choice behaviour slightly more often in smaller and therefore younger females. It could be that the smaller females are less experienced with reproduction and therefore more stressed during the breeding season (Reading, 2001; Romero-Diaz et al., 2019), they might show differences in hormonal mechanisms (Wilczynski and Lynch, 2011) or have less energy reserves after hibernation to struggle with several males. In the following three paragraphs, we will discuss and interpret the behaviour displayed.

Rotation of the female body was the behaviour most often displayed when a female was grabbed by a male (83%). The male was holding against the females movement , using his foot to counter-balance the rotation in order to keep the female tight in amplexus. We observed rotating of couples frequently in the field, which was described by Savage (1934) before. In Darwin´s frog (*Rhinoderma darwinii*), females are kicking the males to test their strength which should correlate with their fathering ability and females prefer larger males (Busse, 2003). This sampling of potential mates can be cost intensive but time effective, which allows females to sample more potential mates during a short breeding season. Fighting or struggling for females by

males is seen as a trial of strength (Savage, 1961). Therefore, the rotating by females might be a sampling tactic to test the male's strength and endurance. If the male can kick-off rivals and prevents the formation of so-called "mating balls", females could increase their survival probabilities when mating with a stronger male. When trapped in a mating ball, the females could easily drown during the forceful attempts of several males (Davies and Halliday, 1979; Howard, 1980).

Smaller females, in relation to the male, were slightly more successful in escaping amplexus while showing rotating behaviour. This difference might be due to a simple mechanistic reason. If females are smaller than males, the female could more efficiently escape the males grip, because males cannot hold tight properly. In cane toads, *Rhinella marina*, it was shown that males with shorter arm length could cling better to females compared to males with longer forearms that were replaced more efficiently because they cannot hold the females properly (Clarke et al., 2019). In the European Common toad, *Bufo bufo*, takeovers from males were more successful when pairs were not size assorted, because a size mismatch leads to a weak binding in amplexus (Höglund, 1989). This is in line with our finding that an increase in size difference towards smaller females increased the escape probabilities. An increase in mechanical grip properties of pairs could add to the probability of encountering size-assortative mating in this population (Dittrich et al., 2018).

During our experiments, females emitted two different types of presumed release calls, a grunting and a squeaking call (paper 2). They contracted their trunk muscles, which forces air through the larynx into the buccal cavity (Wells, 2007), which is exactly the same procedure males apply to produce the acoustically very similar release calls. Apart from the overall acoustic similarity, the female grunting call (paper 2) matches the dominant frequency (300 kHz) of the male release call (Brzoska et al., 1977). We thus find it plausible to assume that the female grunting sound may imitates the male release call and therewith leads to a higher escape chance be the female. Savage (1961) described that the females gave this grunting sound mostly when clasped after egg deposition. This would help females to leave the pond non-harassed and protect the males from wrong decisions. However, in our study all females had not yet deposited their eggs. We thus assume that this sound may have two functions, one is to reject potential mating partners before reproduction (mate choice), and the other is to leave the pond non-harassed after reproduction. Brzoska et al. (1977) described the higher frequency call as the female release call and we termed it the squeaking sound, which seems to be less effective in escaping a male. This sound consist of several frequency bands and therefore seems to be an unspecific signal for a variety of receivers. The broader frequency could be interpreted as a distress call, which usually is used to repel predators (Toledo et al., 2009). During our fieldwork, we sometimes evoked release calls by handling the females, presumably resembling the male grip. However, for clarification of the underlying intentions, better sound recordings and behavioural test are necessary.

Feigning death can be found in many different taxa, from invertebrates to vertebrates (Humphreys and Ruxton, 2018), where it is mainly used as a defensive behaviour against predation (Toledo et al., 2010). It is a stress response to an immediate threat of predation or a strong tactile stimulation and regarded as an "evolutionary conserved defensive mechanism of last resort" (Rogers and Simpson, 2014). Feigning death as a tactic to avoid mating, reproductive cannibalism or male harassment, was so far described in insects and spiders (Bilde et al., 2006; Khelifa, 2017), and only one amphibian species, the sharp-ribbed newt, *Pleurodeles waltl* (Janssenswillen and Bossuyt, 2016). Savage (1961) mentioned that one *R. temporaria* female was feigning death for two hours after being grabbed by a male, and that the male was calling frequently during this time, but did not let her go. We observed this female behaviour frequently, and more often in smaller and therefore younger females (although differences not being statistically significant), which could be due to a lower ability of active resistance or fleeing probability in early life-history stages, when the probability to display death feigning behaviour is higher (Humphreys and Ruxton, 2018). Smaller females could be less experienced during reproduction and the formation of "mating balls" can be observed frequently. In this case, it could be beneficial for the female to feign death instead of rotating her way out of amplexus and thereby provoke attention of other males nearby. Savage (1934) also described that death feigning was displayed when females were grabbed from the ventral side, which would be probably the case when they are already in amplexus with another male. In our study, we excluded intrasexual competition, which could influence the abundance of mate choice behaviour. All the females that feigned death were grabbed from the back in our experiments. Therefore, we consider death feigning not only as an exit strategy, but also as the less energy-consuming tactic either to get rid of an un-wanted male, or to proof their endurance.

We need to ask what could be the (phenotypic) trait under selection that leads to acceptance or resistance of a mating partner and what are the benefits (and costs) of female choice in this species? There are, at least, no obvious direct benefits as parental care or resources defended by the male. Additionally, indirect benefits, as higher fertilization success by larger or size assorted males can be neglected (Dittrich et al., 2018). Nevertheless, we found additive genetic effects of multiple paternity in *R. temporaria* considering developmental time off offspring (Dittrich et al., 2019). Therefore, genetic benefits could increase survival probabilities of offspring and could justify costs associated with mate choice. Future studies should take more variable individuals and different densities into account to increase associated costs and present a variability of potentially preferred phenotypical traits. Contrary to most literature, we show that females in explosive breeding species are not passive during reproduction and do have a freedom to choose their mates. Still we do not know the preferred traits that lay behind female mate choice nor their mate choice strategy, although Savage wrote in his book from 1961 "it is the female that is really in charge of the events".

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Paper 4

Matriline effects on metamorphic traits in a natural system in the European common frog (*Rana temporaria*).

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ORIGINAL RESEARCH

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Matriline effects on metamorphic traits in a natural system in the European common frog (*Rana temporaria*)

Carolin Dittrich[1] | Juliane Huster[2] | Mark-Oliver Rödel[1,3] | Heike Feldhaar[2]

[1]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

[2]Animal Ecology I, Bayreuth Center for Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany

[3]Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

Correspondence
Carolin Dittrich, Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany.
Email: carolin.dittrich@mfn.berlin

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Abstract

Successful reproduction is an important determinant of the fitness of an individual and of the dynamics of populations. Offspring of the European common frog (*Rana temporaria*) exhibit a high degree of variability in metamorphic traits. However, environmental factors alone cannot explain this phenotypic variability, and the influence of genetic factors remains to be determined. Here, we tested whether the maternal genotype influences developmental time, body size, and body condition of offspring in a forest pond in Germany. We collected fertilized eggs from all 57 clutches deposited in the pond. We used multilocus genotypes based on seven microsatellite loci to assign metamorphosed offspring to mothers and to determine the number of fathers for a single matriline. We tested the influence of genetic effects in the same environment by comparing variability of metamorphic traits within and between full-sib offspring grouped to matrilines and tested whether multiple paternity increases the variability of metamorphic traits in a single matriline. The variability in size and body condition was higher within matrilines than between them, which indicates that these traits are more strongly influenced by environmental effects, which are counteracting underlying genetic effects. The developmental time varied considerably between matrilines and variability increased with the effective number of fathers, suggesting an additive genetic effect of multiple paternity. Our results show that metamorphic traits are shaped by environmental as well as genetic effects.

KEYWORDS

amphibians, genetic effects, microsatellites, multiple paternity, natural selection

1 | INTRODUCTION

Natural selection shapes life-history traits of individuals, which optimizes fitness in a given environment. Under optimality theory, the timing of specific life-history events, such as reproduction or metamorphosis, will evolve to an optimum due to natural selection and local adaptation (Parker & Smith, 1990). Metamorphosis, as an important life-history event, has received a lot of attention, with different models developed to explain the best possible transition and niche shift under different environmental conditions in complex life cycles (Rowe & Ludwig, 1991; Rudolf & Rödel, 2007; Wilbur & Collins, 1973).

In natural amphibian populations, the metamorphic traits of individuals (e.g., size, body condition, age at metamorphosis, and developmental time) can differ profoundly within and between populations (Grözinger, Thein, Feldhaar, & Rödel, 2014; Loman, 2004). Factors known to influence these traits under laboratory conditions are temperature, food availability, intra- and interspecific competition, presence of predators, and seasonal time constraints (Drakulić

et al., 2016; Laugen, Laurila, Räsänen, & Merilä, 2003; Laurila & Kujasalo, 1999; Merilä, Laurila, Pahkala, Räsänen, & Laugen, 2000; Pakkasmaa & Aikio, 2003; Smith-Gill & Berven, 1979; Van Buskirk, 2017). Little is known of the interacting effects in natural environments (Loman, 2001, 2004), where environmental variables often seem to counteract the genetic effects. This process, known as countergradient variation, can occur on small geographical scales (Conover & Schultz, 1995; Dittrich, Drakulić, Schellenberg, Thein, & Rödel, 2016; Laugen et al., 2003; Skelly, 2004). Genetic effects have been shown to influence metamorphic traits mainly by high dominance and additive effects, particularly, age at metamorphosis and growth rate (Laugen et al., 2005; Laurila, Karttunen, & Merilä, 2002). In addition, maternal effects like egg size or egg provisioning, could influence metamorphic traits, but were shown to be mostly weak (Laugen et al., 2005) and seem to be highly dependent on the environment. Furthermore, females are able to follow different life-history strategies concerning the age and/or size of first reproduction and could adjust their strategies throughout their reproductive lifetime. Females may allocate their reproductive investment either into a larger quantity (many but small offspring) or quality (fewer but larger offspring) of progeny ("offspring number-size trade-off"; Smith & Fretwell, 1974; Charnov & Ernest, 2006).

The European common frog (*Rana temporaria* Linneaus, 1758) is one of the most widespread amphibians in Central and Northern Europe (Sillero et al., 2014). This generalist species expresses high variability and phenotypic plasticity in metamorphic traits (Grözinger, Feldhaar, Thein, & Rödel, 2018; Laurila, Pakkasmaa, & Merilä, 2001; Ryser, 1996; Ståhlberg, Olsson, & Uller, 2001).

Additionally, multiple paternity was shown to occur in this species, either as a consequence of stray sperm (Laurila & Seppä, 1998) or of "clutch piracy" (Vieites et al., 2004). Multiple paternity could increase genetic variability among offspring and thereby increase viability of offspring (Jennions & Petrie, 2000). In laboratory studies, a sire effect on developmental time and survival was found (Laugen, Laurila, & Merilä, 2002; Merilä, Laurila, Pahkala, et al., 2000). In this study, we investigate the influence of maternal genotypes and putative effects of multiple paternity on post-metamorphic traits and trait variability within one natural pond. To our knowledge, the assignment of anuran metamorphs to their respective matrilines with molecular techniques is unique and the first study of its kind. All individuals share the same environment and therefore environmental effects, which could influence metamorphic traits. Microsatellite analysis was used to assign full- and half-siblings to a single mother (matriline) and determine the number of fathers. Furthermore, we examined the effect of multiple paternity on the variability of metamorphic traits of the progeny within matrilines.

We tested the following hypotheses:

1. Offspring from different mothers show high variability in metamorphic traits between matrilines within one shared environment, due to maternal and paternal genetic effects.
2. An increased number of sires of one clutch should increase the variability in metamorphic traits within the respective matriline due to additive genetic effects.
3. Some matrilines are more successful in reproduction than others due to faster offspring development, bigger offspring and higher offspring numbers in the same environment, due to different resource provisioning.

2 | MATERIAL AND METHODS

2.1 | Site and sampling of clutches

Clutch samples and metamorphs of *R. temporaria* were collected from a pond in the northern Steigerwald (Bavaria, Germany), near the village of Fabrikschleichach (49°54′N, 10°32′E). From the 1970s, 120 small artificial ponds were constructed in this 28 km^2 area for conservation purposes by the state forestry department. Our study pond has a surface of 12 m^2 and is located in a 28 km^2 beech grove and mixed forest which has been monitored for *R. temporaria* breeding sites since 2005 (Grözinger, Wertz, Thein, Feldhaar, & Rödel, 2012). The maximum depth of the surplus water in the middle of the pond is approx. 50 cm throughout the year. Clutches were deposited within the patchy vegetation on the shallow southern part of the pond (Supporting Information Figure S1). The water temperature was measured with a Thermochron iButton© (accuracy ±0.5°C), and average daily values are given in Supporting Information Figure S2. Additionally, data on local precipitation were obtained from a weather station 2.5 km from the study pond (Supporting Information Figure S2). The pond was checked daily for new clutches from 1st of April until 12th of April. Although the first clutches were already found on 1st of April, we believe that these embryos experienced only marginal (if any) developmental advantage, due to an unusual cold period from 1st to 8th of April. In this period, maximum daily temperatures reached 3°C, at which developmental progress ceases (Loman, 2002). The first hatchlings were observed on 20th of April. During the yearly monitoring of clutches from 2005 to 2018, we found a range of 19 to 103 clutches per year for this specific pond. In the close surroundings (500 m radius), 21 ponds are present, six of which are regularly used for spawning by the common frog (in more than six out of 13 years). The first 30 clutches of *R. temporaria* were found on 1st April 2013, the last clutches were deposited on 12th April (total $n = 57$). We sampled 10 eggs each from all clutches and kept them in small plastic containers (Ø 6 cm, 7 cm high) for 48–72 hr at 8°C until the embryos reached Gosner stage 17–20 (Gosner, 1960). Afterward, the embryos were stored in 99% ethanol until further use.

2.2 | Sampling of metamorphs

To intercept all emerging metamorphs, a fence was installed at the beginning of June 2013 encircling the pond entirely. As soon as metamorphs began leaving the pond, the fence was controlled twice daily (from 8th July to 29th August 2013). Up to 50 metamorphs were captured each day and two measurements were taken: (a) body mass, measured with an electronical balance to the nearest 0.05 g (VOLTKRAFT PS 250) and (b) snout-vent length (SVL), measured on

scale paper with millimeter grid to the nearest 0.5 mm. Two DNA samples were taken by gently swabbing the skin with cotton buds. DNA samples were stored in 1.5 ml reaction tubes containing either 300 µl Cell Lysis Solution (CLS; PUREGENE® DNA Purification Kit; Qiagen) or 300 µl 99% EtOH. All metamorphs were released in a wet area outside the fence. If more than 50 metamorphs emerged per day, measurements were taken from 50 randomly chosen individuals, and all other metamorphs were only counted and released immediately.

2.3 | Microsatellite analyses

DNA was isolated from four eggs per clutch (n = 232 in total) and from 1,176 metamorphs (a maximum of 30 per sampling day) using the PUREGENE® DNA Purification Kit (Qiagen) and stored at −20°C until further use. Individuals were genotyped using microsatellite markers. The microsatellite DNA was amplified via polymerase chain reaction (PCR; details in Supporting Information Table S1) in a total reaction volume of 12.5 µl. We used seven specific primers pairs (BFG046, BFG090, BFG099, BFG203, BFG237, BFG242, BFG250; Matsuba & Merilä, 2009), which were labeled with a fluorescent dye (details in Supporting Information Table S2).

PCR products were analyzed via polyacrylamide gel electrophoresis with a LI-COR 4300 DNA Analyser (LI-COR Biosciences). Alleles were scored with SAGA™ GENERATION 2 automated microsatellite software (LI-COR Biosciences) and revised manually.

MICRO-CHECKER version 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) was used to test for null alleles, scoring errors, and large allele dropout. Genotypes with at least five out of seven loci scored (n metamorphs = 706) were used for the detection of scoring errors and overall homozygote excess.

For sibship analysis, we used the software COLONY version 2.0.6.3 (Wang, 2004). The software is based on full-pedigree likelihood methods to infer sibship among individuals by using multilocus genotype data (Jones & Wang, 2010). Each female is considered to spawn only one clutch per season (Savage, 1961), and we used the genotypes of the clutch samples as additional input to improve sibship assignment as larvae from one clutch represent maternal sibs. The length of the run was set to medium, inbreeding was excluded, and the mating system was set to polygamy for females, because a high proportion of multiple paternity has been shown for *R. temporaria* (Laurila & Seppä, 1998). Offspring sired by the same father but different mothers (half-sibs) could be genetically more similar than siblings from another matriline; therefore, the mating system for males was set to monogamy to increase differences between matrilines. The allele dropout rate was set to 0.01%, except for the loci BFG046 and BFG242 where a former run of COLONY estimated dropout rates around 0.05%. Allelic dropout occurs when the PCR fails to amplify one of the homologues genes at a locus and therefore could lead to false homozygotes, which could influence the grouping of an individual into a sibship (Wang, 2004). The marker error rate was set to 0.01% for all loci, because these types of errors (false alleles, mutations or contaminant DNA) are less frequent (Wang, 2004). The software arranged the samples of clutch and metamorphs to clusters with a probability of sibship ranging between 0 and 1. Clusters with a probability higher than 0.8 were used for further analysis and defined to represent offspring of a matriline. Some clusters were grouped without clutch sample, which could be due to allelic dropouts that may occur due to the low DNA concentrations we used (Gagneux, Boesch, & Woodruff, 1997). To compare variance in phenotypic traits of offspring within and between matrilines, we only used clusters comprising at least six full-sibs for further analysis.

2.4 | Statistical analyses

2.4.1 | All emerging metamorphs

All statistical analyses were performed using R statistical software (R Core Team, 2018). To investigate a potential relationship between the SVL and mass of all emerging metamorphs, a Pearson correlation and regression analysis was performed. Because the main spawning time comprised only a few days, all embryos started their development at approximately the same time. Therefore, developmental time was calculated and defined as the time from the beginning of development (median date of spawning activity 10th April 2013) until the end of metamorphosis (day the respective metamorph was collected at the fence). We calculated the body condition index (BCI; scaled mass index after Peig & Green, 2009) of metamorphs. The exponent to calculate the BCI (3.08) was taken from Drakulić et al. (2016), as they studied the same *R. temporaria* population. The measure of body condition gives insights on how well metamorphs are provided with resources to increase the probability of future survival (Scott, Casey, Donovan, & Lynch, 2007). We tested the relationship of SVL and BCI with developmental time using generalized additive models (GAM), because assumptions for linear regression analyses were not met. The models were fitted with restricted maximum likelihood method, and cubic regression splines were used for the explanatory variables SVL and BCI. The GAMs were calculated with the R package MGCV (version 1.8-24; Wood, 2011). All graphs were drawn with R package GGPLOT2 (version 3.0.0; Wickham, 2009), and the "jitter" function was used to avoid overplotting.

2.4.2 | Multiple paternity and differences in metamorphic traits

To investigate the rate of multiple paternity, we used the mating frequency defined as number of fathers per matriline. To examine the relative proportion of offspring sired by a male, the effective mating frequency (m_e) was calculated (Starr, 1984).

Multiple paternity increases the genetic variability among the offspring of a matriline (Jennions & Petrie, 2000). The influence of multiple paternity on variability in metamorphic traits of the offspring was investigated by comparing two datasets. One dataset ("main father") contained metamorphs of the main father only (full-sibs), which we defined as the father who was represented in the clutch sample or in clusters without clutch samples, the father with

the highest number of offspring. The second dataset ("all fathers") contained all metamorphs from all fathers of a matriline (full and half-sibs).

We used a paired *t* test to investigate whether multiple paternity changes the mean SVL, mean BCI or mean developmental time within matrilines. The coefficient of variation (CV) was used as a measurement of variability of these traits within a matriline. The CV of SVL, BCI, and developmental time for metamorphs from main father and all fathers were calculated for each matriline and compared with a paired *t* test. Single-mated matrilines were excluded from this analysis. To correct for the different number of metamorphs from main and all fathers of the same matriline, which could affect detected changes in mean or CV due to larger sample size in the all father dataset, we randomly subsampled the same number of metamorphs from main and all fathers 10 times.

2.4.3 | Differences in metamorphic traits between matrilines for full-sibs

After assigning metamorphs to matrilines, we tested if SVL, BCI, and developmental time of full-sibs (with the same broad genotype) differ between matrilines using the Kruskal–Wallis rank sum test. If metamorphic traits showed significant differences, we performed a post hoc analysis using the Dunn test with *p*-value correction for multiple testing (false discovery rate; Benjamini & Hochberg, 1995) using the R package FSA (version 0.8.20; Ogle, 2017).

2.4.4 | Influence of number of offspring and number of fathers on metamorphic traits

Due to resource partitioning (Smith & Fretwell, 1974), we tested if the number of successfully developing progeny could be related to metamorphic traits, for example, that numerous offspring from one matriline is especially small or large in SVL. If applicable, we used a linear model to see which variables have an influence on mean size and mean BCI of metamorphosed offspring from single matrilines, with number of progeny, number of fathers, and mean developmental time as explanatory variables for the whole dataset (full- and half-sibs). If assumptions for linear regression were not met, we used GAMs.

3 | RESULTS

3.1 | Emigration pattern of *R. temporaria* metamorphs

Overall, 2,414 metamorphs emerged during the whole emigration period (8th July to 29th August 2013). The maximum number of individuals leaving the pond per day was 118 (Figure 1). Given that a clutch contains on average 1,117 eggs (Grözinger et al., 2014) and that we sampled 57 clutches, the survival rate from egg to metamorphosis was 3.8%. Developmental time between metamorphs was highly variable. The majority of the metamorphs (n = 1,753; 72%) left within the first 3 weeks of the migration period until day 112 (31st July 2013). The last 28% (n = 676) left within the last 4 weeks of the migration period with daily numbers of metamorphs continuously decreasing (Figure 1).

3.2 | SVL, body condition index, and developmental time of all emigrating metamorphs

We measured the SVL, metamorphic mass, and the day of emigration of 1,943 metamorphs (maximum 50 metamorphs per day). The relationship of size and mass was following a nonlinear relation and can be described best by a raw quadratic polynomial function of size on mass (Figure 2; mass = 0.26 − 0.04 × size + 0.003 × size^2, df = 1,940, p < 0.001, adjusted R^2 = 54%). The calculated GAM for metamorphic size as response to developmental time showed a significant, but nonlinear influence of time (SVL increases with time until day 105 and decreases after day 118, Supporting Information Figure S3). Developmental time explained 12.4% of variance in SVL. The GAM for BCI as response to developmental time showed a significant, but nonlinear influence of time (BCI reaches a maximum around day 110,

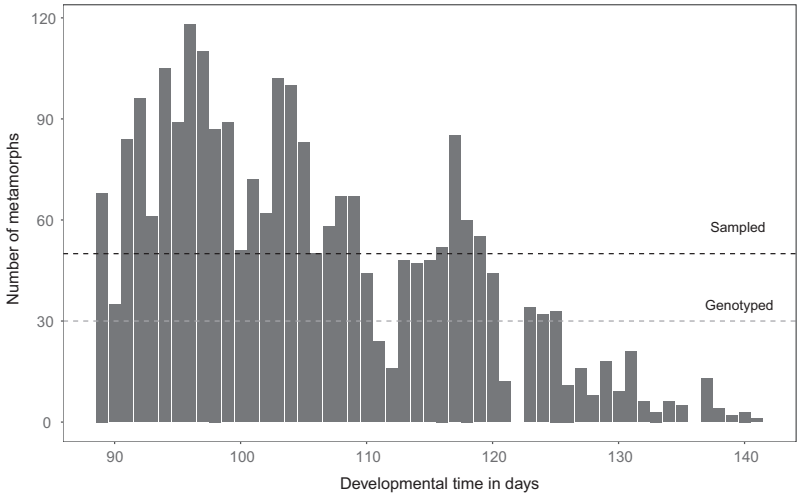


FIGURE 1 Emigration pattern of *Rana temporaria* metamorphs from one pond in 2013 (8th July–29th August 2013). The black dashed line marks 50 metamorphs (maximum number of sampled metamorphs per day) and the gray dashed line marks 30 metamorphs (maximum number of genotyped metamorphs per day). Missing bars are days without sampling

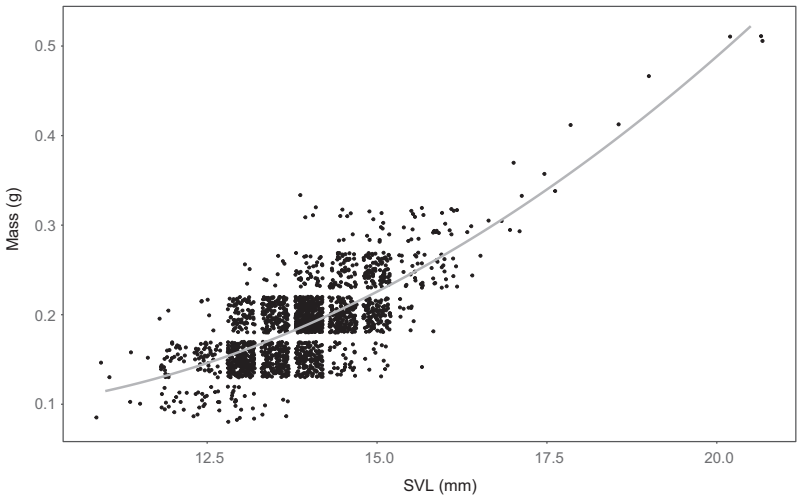


FIGURE 2 Relationship of SVL (mm) and metamorphic mass (g) of *Rana temporaria* metamorphs (n = 1,943) with raw quadratic polynomial function of size to mass (mass = 0.26–0.04 × size + 0.003 × size^2, gray line). Data points are jittered to avoid overplotting

Supporting Information Figure S4) that explained 8.5% of the observed variation in BCI.

3.3 | Sibship/matriline analyses

To improve the assignment of metamorphs to single matrilines, we genotyped four embryos from each clutch (n = 57) and used them as known maternal sibs. From the 2,414 metamorphs that emigrated from the pond, we genotyped 1,176 (maximum 30 per day). In total, 706 metamorphs with five (191 individuals), six (284 individuals), and seven (231 individuals) scored polymorphic microsatellite loci were used for sibship assignment in COLONY software and the number of alleles ranged from 14 to 25 alleles per locus (Supporting Information Table S3).

The COLONY software computed 67 clusters based on multilocus genotypes. Ten of these clusters were excluded, because the probability of sibship within the cluster was too low (<0.8). An additional seven clusters were excluded because samples of two or more clutches were clustered together, which could be due to relatedness of spawning females. Of the remaining 50 clusters, 23 were generated without clutch samples. As defined above, a cluster without a clutch sample contained at least six metamorphs from the same father genotype to be designated as a matriline. Thus, 10 of these 23 clusters were excluded. The remaining 40 clusters were defined as matrilines and were used for further analyses (n = 439 metamorphs). More details can be found in Appendix S1.

3.4 | Multiple paternity and differences in metamorphic traits

Only eight matrilines exclusively contained full-sibs, and 32 of all 40 matrilines were fertilized by multiple males and therefore contained

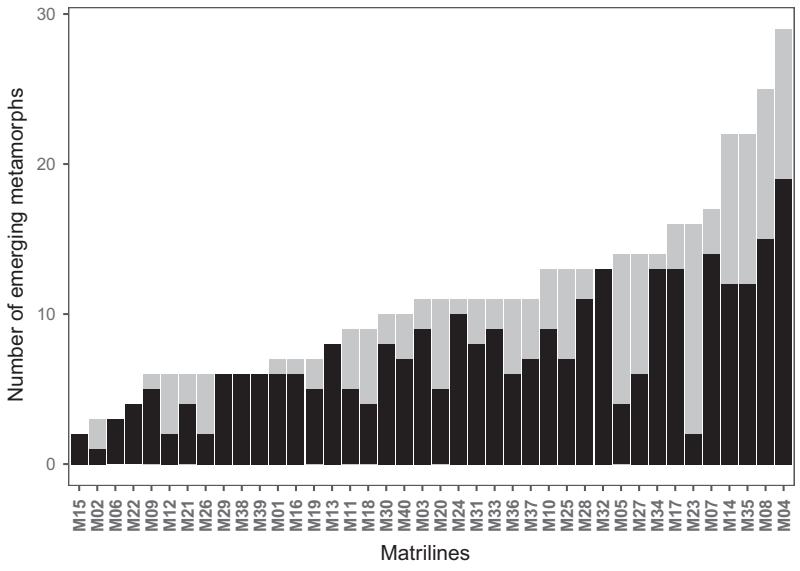


FIGURE 3 Number of metamorphs per matriline from main father (black, n = 294) and all fathers (gray, n = 439)

TABLE 1 Comparison of metamorphic traits of offspring from main and all fathers within a matriline

Metamorphic trait	Mean	Subsampling (significant/not significant)	CV	Subsampling (significant/not significant)
SVL	t = −2.4663, df = 31, p = 0.01938, d = 0.44	3/10	t = −2.2049, df = 30, p = 0.03527, d = 0.4	1/10
BCI	t = 0.10494, df = 31, p = 0.9171, d = 0.02	0/10	t = −2.3403, df = 30, p = 0.02611, d = 0.42	3/10
Developmental time	t = 0.92399, df = 31, p = 0.362; d = 0.16	0/10	t = −4.6786, df = 30, p < 0.001, d = 0.84	10/10

Note. Given is a paired t test and Cohen's d as effect size for changes in mean values, changes in coefficient of variation (CV) and results from the subsampling to correct for different numbers of offspring from main father and all fathers.

half-sibs (Figure 3). We found a mean mating frequency of 2.7 fathers per matriline, with a range from one to five fathers. Additionally, we calculated the weighted average of fathers per matriline, called effective mating frequency (m_e) that had a mean value of 1.8 and was smaller than the mean mating frequency. This shows that not all fathers sired an equal number of offspring per matriline. The main father per matriline sired 1 to 19 offspring (mean ± *SD* = 7 ± 4; total *n* offspring main fathers = 294), and all fathers together per matriline sired 1 to 29 offspring (mean ± *SD* = 11 ± 6; total *n* offspring all fathers = 439) (Figure 3).

Multiple paternity, and therefore higher genetic variability, could lead to differences in metamorphic traits within matrilines. We conducted paired *t* tests to compare the mean values of metamorphic traits of progeny within one matriline regarding single or multiple paternity. While the mean values of BCI and developmental time within matrilines were not changed by multiple paternity, we detected an increase in SVL with multiple paternity (Table 1). However, the differences were not supported by random subsampling of the matrilines. We conclude that the significant difference in mean SVL was due to a larger number of offspring in the all father dataset and does not represent a real effect based on multiple paternity.

To investigate whether variability of metamorphic traits was influenced by multiple paternity, we compared the coefficients of variation (CV) for both datasets.

The variability in all metamorphic traits was increased by multiple paternity when comparing main and all fathers (Table 1), but only developmental time was significantly more variable for offspring from all fathers (mean ± *SD*: 7.8 ± 3.0) than for offspring from main father (mean ± *SD*: 5.4 ± 1.8; Table 1) after random subsampling of metamorphs. This is supported by a large effect size (Cohens d = 0.84). Additionally, we detected a positive correlation of effective number of fathers and the CV in developmental time (Pearson correlation: r = 0.44, CI 0.15–0.66, p = 0.004), but not in the other metamorphic traits.

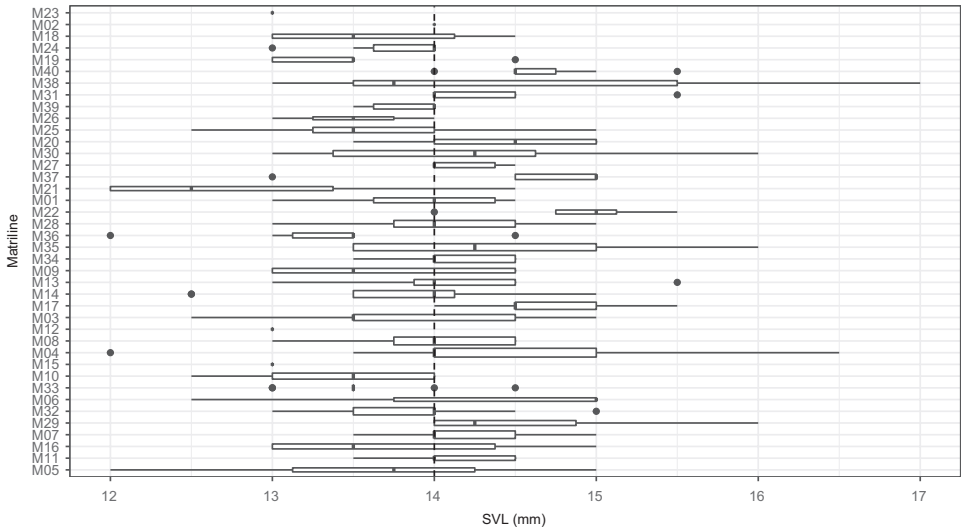
3.5 | Differences of metamorphic traits between matrilines

We tested if metamorphic traits differ between matrilines and therefore used the main father dataset of 295 individuals assigned to 40 matrilines (same broad genotype per matriline). The median SVL of metamorphs differed significantly between matrilines (Kruskal–Wallis rank sum test: χ^2 = 84.89, df = 39, p < 0.0001), indicating a genetic or maternal effect. Overall, SVL of individuals ranged from 12 to 17 mm. The median of all individuals was 14 mm. The median SVL of the different matrilines ranged from 12.5 to 15 mm (Figure 4). After a post hoc Dunn test, we found that only two out of 780 comparisons between matrilines were significantly different concerning their SVL. Offspring from M17 had significantly bigger individuals (median 14.5 mm) than M10 and M33 (both median 13.5 mm; detailed results of Dunn test in Supporting Information Table S4). Nevertheless, in most comparisons, within matriline variability in SVL was higher than between matriline variability measured by the CV (range CV: 1.8–11.1, Table 2).

We found no significant difference between the median BCI of matrilines (Kruskal–Wallis rank sum test: χ^2 = 51.89, df = 39, p = 0.08107). Overall, BCI of individuals ranged from 0.11 to 0.30 with a median of 0.20. The median BCI of the different matrilines ranged from 0.16 to 0.23 (Figure 4). BCI was less variable between matrilines, but showed a high variability within matrilines (range CV: 4.6–21.2, Table 2).

The developmental time differed significantly between matrilines (Kruskal–Wallis rank sum test: χ^2 = 226.21, df = 39, p < 0.0001), indicating a genetic or maternal effect. Overall, developmental time of individuals ranged from 89 to 140 days, with a median of 106 days. The median developmental time of the different matrilines ranged from 91 to 135 days (Figure 4). After a post hoc Dunn test, we found that 267 of 780 comparisons between matrilines were significantly

FIGURE 4 Snout-vent length (SVL in mm), body condition index (BCI), and developmental time (days) of emigrated full-sib metamorphs for each matriline. The dashed lines represent the overall median of size (14 mm), body condition (0.2013), and developmental time (107 days). Matrilines are ordered from short to long developmental time in each plot. The developmental time does not correlate with SVL or BCI. Box whisker plot: The box goes from 25th percentile to 75th percentile of the data. The line in the box indicates the median and the whiskers extending to the furthest data point that is within 1.5 times the box. Data points past the ends of the whiskers are considered outliers and are shown as black dots. The width of the box is proportional to the number of metamorphs per matriline


Matriline
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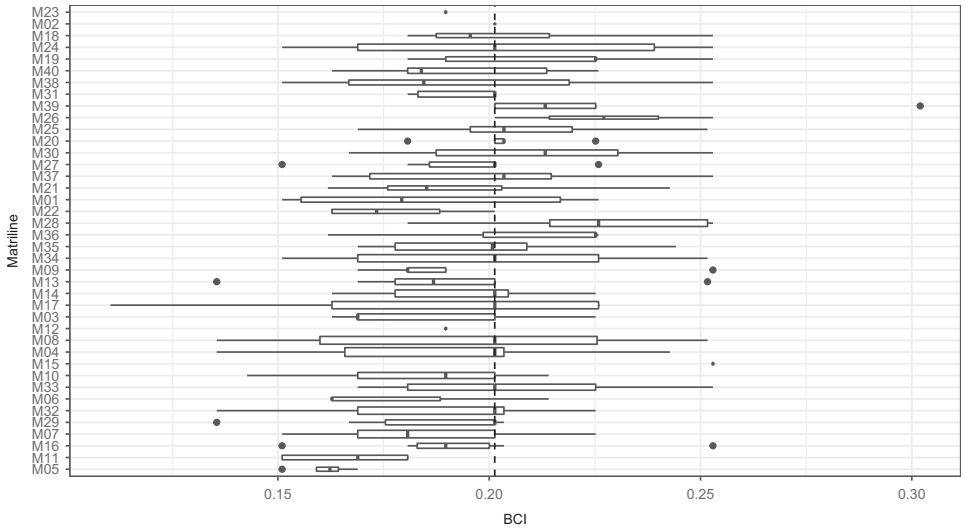
17
SVL (mm)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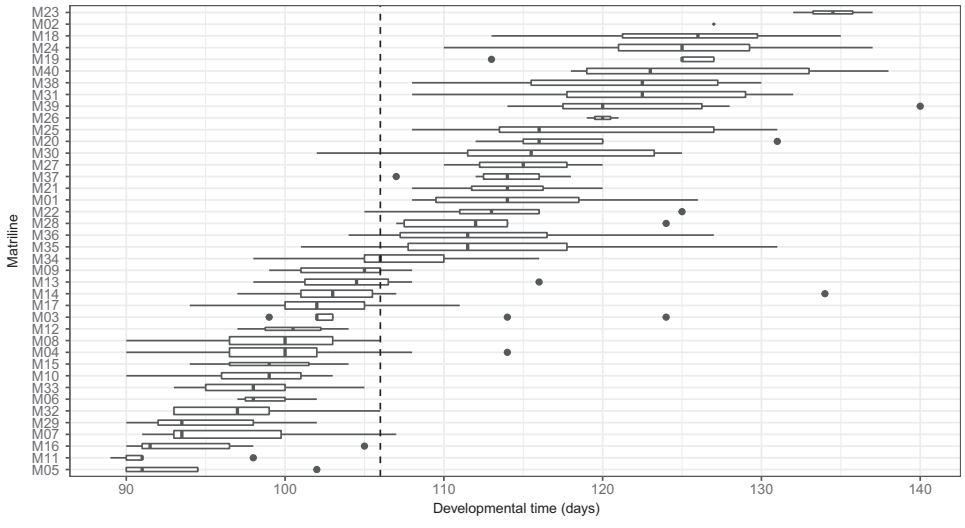
BCI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M05
90
100
110
120
130
140
Developmental time (days)

different concerning their developmental time. For example, offspring of M11 (median 91 days) had a significantly shorter developmental time than offspring of M23 (median 135 days; results of Dunn test in Supporting Information Table S5). In fact, all metamorphosing offspring of M11 had left before offspring of M23 had started to leave the pond. Developmental time showed the highest variability between matrilines, but also high within-matriline variation (range CV: 1.2–9.1, Table 2).

3.6 | Influence of number of offspring and number of fathers on metamorphic traits

Life-history strategies could differ between mothers, where some invest in a small number of eggs with higher amount of resources than average, or they invest in a large number of eggs with a lower amount of resources than average. The amount of resources in the egg should be positively correlated with the body size at metamorphosis.

The linear model of mean SVL per matriline, $F(3,36) = 2.909$, $p = 0.04771$, $R^2 = 0.13$, suggested that developmental time did not influence SVL ($\beta = 0.0086$, $p = 0.2592$), but that number and size of offspring was positively related ($\beta = 0.0367$, $p = 0.0125$). This indicates that some matrilines had more metamorphosing offspring with larger SVLs (Figure 5). Interestingly, the number of fathers had a negative influence on size ($\beta = -0.1817$, $p = 0.0125$; Figure 5).

Additionally, we ran the same model with effective number of fathers to account for the different proportion of offspring sired; however, none of the variables influenced SVL (model in Appendix S2). Nevertheless, the trend in the data was the same, with number of offspring having a positive influence and effective number of fathers a negative influence.

The BCI could not be fitted to a linear model, as assumptions were not met. Instead, we used a GAM. Neither number of offspring, nor number of fathers (or effective number of fathers) had an influence on the mean BCI of matrilines, when included as smoothed terms. The developmental time showed a positive albeit not significant linear trend ($\beta = 0.0005$, $p = 0.095$), indicating a higher BCI per matriline with longer pond development. The summary of the GAM can be found in the Appendix (S3, Supporting Information Figure S5).

4 | DISCUSSION

Our hypotheses were that high variability in metamorphic traits between offspring from different matrilines developing in the same environment should be influenced by maternal and paternal genetic effects. Additionally, multiple paternity should increase genetic variability of offspring within matrilines, therefore increasing variability in metamorphic traits of *R. temporaria*. We found low variability between matrilines in size and body condition, but high variability in developmental time in the same environment, potentially due to genetic effects. In addition, multiple paternity seems to be very common and increased the variability of developmental time, but not the variability of other metamorphic traits. Additive genetic effects of fathers seem to act on developmental time, because we found a positive relationship of the effective number of fathers and the variability in developmental time within single matrilines. Additionally, the number of metamorphosed offspring differed between matrilines, with some having a higher number of progeny with larger SVL, indicating that some *R. temporaria* females reproduce more successfully than others in the same environment.

4.1 | Overall emigration pattern and relationship of size and BCI with developmental time

The size and BCI of all emerging metamorphs was only marginally influenced by developmental time and did not show a linear relationship. Consequently, there was almost no difference in size or BCI of metamorphs with a short or a long developmental time. These findings contrast with former models on amphibian metamorphosis (e.g., Wilbur & Collins, 1973). However, environmental stress, such as food shortage, decreasing water level, and predation or density effects (exploitative or interference competition) in a natural environment, could lead to a negative relationship of size and age of metamorphosis (Laurila et al., 2001; Merilä, Laurila, Laugen, Räsänen, & Pahkala, 2000; Relyea, 2007; Wong, Griffiths, & Beebee, 2000). The multiple biotic and abiotic influences acting in parallel in our natural system may have led to trade-offs between growth and development (Laugen et al., 2003; Loman, 2016) and could counteract potential underlying intrinsic genetic effect (Conover & Schultz, 1995). Former studies on the same natural population showed similar patterns (Grözinger et al., 2018, 2014), where environmental factors alone could not explain the variation in observed metamorphic traits.

4.2 | Differences of metamorphic traits between matrilines of *R. temporaria*

We assumed that the variability observed in former studies (Grözinger et al., 2018, 2014) might be due to differences in resource allocation or intrinsic genetic effects within single matrilines. Indeed, when we assigned metamorphs to their respective matrilines, we detected differences in metamorphic traits among offspring from different mothers. We observed the most profound differences in developmental time, where metamorphs from fast developing matrilines left the pond before individuals from slow developing matrilines even started emigration. Even if the breeding spanned over approximately 12 days, we think that the first clutches could not experience a developmental advantage due to unfavorable weather conditions (Loman, 2002). Therefore, priority effects (Eitam, Blaustein, & Mangel, 2005; Wong et al., 2000) should have limited, if any, effect on developmental time. However, there were fewer differences in SVL and BCI than in developmental time. When exploitative competition occurs and tadpoles of different size classes are competing for limited resources, an intermediate size could be favored (van Buskirk, Cereghetti, & Hess, 2017). This would counteract intrinsic

TABLE 2 Summary values for main father and all fathers' offspring per matriline

Matriline	Number of metamorphs		Number of fathers		m_e	SVL (mm)				Developmental time (days)				Body condition index			
	Main father	All fathers	Within clutch	All		Main father	CV	All fathers	CV	Main father	CV	All fathers	CV	Main father	CV	All fathers	CV
M1	6	7	1	2	1.3	13.9 ± 0.6	4.2	14.0 ± 0.6	4.1	115 ± 7	6.1	115 ± 6	5.6	0.19 ± 0.03	18.6	0.18 ± 0.03	17.1
M2	1	3	2[a]	3	3.0	14.0	NA	14.3 ± 0.6	4	127	NA	113 ± 17	4	0.20	NA	0.22	13
M3	9	11	1	2	1.4	13.9 ± 0.8	5.9	13.9 ± 0.9	6.2	106 ± 8	5.9	111 ± 14	6.2	0.18 ± 0.02	12.9	0.19 ± 0.03	16.4
M4	19	29	2	3	2.0	14.5 ± 1.1	7.4	14.3 ± 1.1	7.7	100 ± 6	6.1	100 ± 8	7.5	0.19 ± 0.03	14.9	0.19 ± 0.03	16.6
M5	4	14	2	4	3.2	13.6 ± 1.3	9.2	14.1 ± 1.1	7.5	94 ± 6	6.1	102 ± 13	13	0.16 ± 0.01	4.6	0.18 ± 0.03	14.3
M6	3	3	1	1	1.0	14.2 ± 1.4	10.2	14.2 ± 1.4	10.2	99 ± 3	2.7	99 ± 3	2.7	0.18 ± 0.03	16.5	0.18 ± 0.03	16.5
M7	14	17	2	2	1.4	14.2 ± 0.5	3.8	14.4 ± 0.7	4.6	96 ± 5	5	97 ± 5	5.1	0.19 ± 0.02	11.4	0.18 ± 0.02	10.6
M8	15	25	1	5	2.3	14.0 ± 0.5	3.3	14.2 ± 1.4	9.8	100 ± 5	4.9	107 ± 12	11.3	0.19 ± 0.04	18.6	0.19 ± 0.03	15.4
M9	5	6	2	2	1.4	13.7 ± 0.8	5.5	13.8 ± 0.7	5	104 ± 4	3.6	103 ± 4	3.4	0.19 ± 0.03	17.2	0.19 ± 0.03	18.6
M10	9	13	2	5	2.0	13.3 ± 0.6	4.6	13.4 ± 0.8	5.7	98 ± 4	4	99 ± 4	4.5	0.19 ± 0.02	12	0.19 ± 0.03	15.5
M11	5	9	2	3	2.3	14.1 ± 0.4	3	14.3 ± 0.9	6	92 ± 4	3.9	93 ± 5	5.3	0.17 ± 0.01	9	0.17 ± 0.02	11.3
M12	2	6	2	3	2.6	13.0	0	13.2 ± 0.5	3.9	101 ± 5	4.9	105 ± 13	12.8	0.19	0	0.20 ± 0.05	22.7
M13	8	8	1	1	1.0	14.1 ± 0.7	5.3	14.1 ± 0.7	5.3	105 ± 6	5.4	105 ± 6	5.4	0.19 ± 0.03	17.4	0.19 ± 0.03	17.4
M14	12	22	1	4	2.3	13.8 ± 0.8	5.5	13.9 ± 0.7	5	105 ± 10	9.1	103 ± 8	7.6	0.19 ± 0.02	10.6	0.19 ± 0.02	8.3
M15	2	2	1	1	1.0	13.0	0	13.0	0	99 ± 7	0	99 ± 7	0	0.25	0	0.25	0
M16	6	7	1	2	1.3	13.8 ± 0.9	6.4	13.7 ± 0.8	5.9	95 ± 6	6.2	96 ± 7	7.7	0.19 ± 0.03	17.2	0.19 ± 0.03	16.8
M17	13	16	1	3	1.5	14.7 ± 0.4	3	14.8 ± 0.4	2.8	102 ± 4	4.3	102 ± 4	3.9	0.19 ± 0.04	21.2	0.18 ± 0.04	20.1
M18	4	9	1	4	3.0	13.6 ± 0.8	5.5	13.9 ± 0.8	5.8	125 ± 9	7.4	123 ± 10	8.2	0.21 ± 0.03	15.7	0.20 ± 0.03	16.2
M19	5	7	1	3	1.8	13.5 ± 0.6	4.5	13.5 ± 06	4.3	123 ± 6	4.8	119 ± 9	7.9	0.21 ± 0.03	13.7	0.21 ± 0.03	12.4
M20	5	11	2[a]	2	2.0	14.4 ± 0.7	4.5	14.2 ± 0.7	4.8	119 ± 7	6.2	110 ± 12	10.6	0.20 ± 0.02	7.8	0.20 ± 0.02	11.8
M21	4	6	1	3	2.0	12.9 ± 1.2	9.2	13.3 ± 1.2	8.8	114 ± 5	4.4	116 ± 5	3.9	0.19 ± 0.03	17.9	0.19 ± 0.03	14.4
M22	4	4	2[a]	1	1.0	14.9 ± 0.6	4.2	14.9 ± 0.6	4.2	114 ± 8	7.2	114 ± 8	7.2	0.18 ± 0.02	10.5	0.18 v 0.02	.5
M23	2	16	2	5	2.3	13.0	0	13.3 ± 0.8	5.7	135 ± 4	2.6	120 ± 8	6.7	0.19	0	0.21 ± 0.03	15
M24	10	11	1	2	1.2	13.8 ± 0.4	2.5	14.3 ± 1.6	11.2	125 ± 8	6.1	123 ± 10	7.8	0.20 ± 0.04	20.3	0.20 ± 0.04	19.8
M25	7	13	1	4	2.5	13.6 ± 0.8	5.9	13.9 ± 0.8	5.5	119 ± 9	7.6	114 ± 12	10.5	0.21 ± 0.03	12.7	0.20 ± 0.03	16.1
M26	2	6	1	3	2.6	13.5 ± 0.7	5.2	13.8 ± 0.6	4.5	120 ± 1	1.2	123 ± 6	4.7	0.23 ± 0.04	16.1	0.19 ± 0.05	25.5
M27	6	14	1	4	2.6	14.2 ± 0.3	1.8	14.0 ± 0.6	4.2	115 ± 4	3.4	110 ± 8	7.4	0.19 ± 0.03	13.1	0.20 ± 0.03	13.9
M28	11	13	NA	3	1.4	14.1 ± 0.6	4	13.9 ± 0.8	5.5	112 ± 5	4.6	111 ± 9	8.5	0.23 ± 0.02	10.7	0.23 ± 0.02	10.2
M29	6	6	NA	1	1.0	14.6 ± 0.8	5.5	14.6 ± 0.8	5.5	95 ± 5	4.9	95 ± 5	4.9	0.18 ± 0.03	15.1	0.18 ± 0.03	15.1
M30	8	10	NA	2	1.5	14.2 ± 1.0	7.3	14.1 ± 1.0	7	116 ± 8	7.2	114 ± 12	10.1	0.21 ± 0.03	14.7	0.21 ± 0.03	14.3

(*Continues*)

TABLE 2 (Continued)

Matriline	Number of metamorphs		Number of fathers		m_e	SVL (mm)				Developmental time (days)				Body condition index			
	Main father	All fathers	Within clutch	All		Main father	CV	All fathers	CV	Main father	CV	All fathers	CV	Main father	CV	All fathers	CV
M31	8	11	NA	4	1.8	14.3 ± 0.5	3.7	14.2 ± 0.5	3.6	122 ± 8	6.8	117 ± 12	10.4	0.19 ± 0.01	5.2	0.19 ± 0.02	9.1
M32	13	13	NA	1	1.0	13.9 ± 0.5	3.7	13.9 ± 0.5	3.7	97 ± 4	3.9	97 ± 4	3.9	0.19 ± 0.03	16.2	0.19 ± 0.03	16.2
M33	9	11	NA	3	1.5	13.6 ± 0.5	3.4	13.6 ± 0.5	3.5	98 ± 4	4	101 ± 9	9.2	0.20 ± 0.03	14.4	0.21 ± 0.03	14.7
M34	13	14	NA	2	1.2	14.1 ± 0.3	2.5	14.1 ± 0.3	2.4	107 ± 5	5.1	106 ± 5	5	0.20 ± 0.03	15.9	0.20 ± 0.03	15.2
M35	12	22	NA	4	2.3	14.3 ± 0.8	5.9	14.5 ± 1.1	7.2	114 ± 8	7.2	113 ± 7	6	0.20 ± 0.02	12.6	0.20 ± 0.03	15.4
M36	6	11	NA	3	2.3	13.3 ± 0.8	6.1	13.2 ± 0.6	4.6	113 ± 8	7.5	111 ± 7	6.7	0.21 v 0.03	13	0.21 ± 0.04	18.5
M37	7	11	NA	3	2.1	14.6 ± 0.7	5	14.5 ± 0.6	4.4	114 ± 4	3.2	115 ± 6	5.1	0.20 ± 0.03	16.7	0.20 ± 0.03	15.3
M38	6	6	NA	1	1.0	14.5 ± 1.6	11.1	14.5 ± 1.6	11.1	121 ± 9	7.1	121 ± 9	7.1	0.19 ± 0.04	20.3	0.19 ± 0.04	20.3
M39	6	6	NA	1	1.0	13.8 ± 0.3	1.9	13.8 ± 0.3	1.9	123 ± 9	7.7	123 ± 9	7.7	0.23 ± 0.04	12.5	0.23 ± 0.04	12.5
M40	7	10	NA	3	1.9	14.6 ± 0.5	3.3	14.4 ± 0.6	3.9	126 ± 8	6.7	127 ± 7	5.7	0.19 ± 0.02	12.5	0.19 ± 0.02	11.2

Note. Shown are the number of metamorphs (for main and all fathers), the number of fathers that sired offspring (found within the clutch and all offspring), the effective mating frequency (m_e), the size of metamorphs (SVL in mm), developmental time (days), and the body condition index. Metamorphic traits are given as mean ±SD and the corresponding coefficient of variation (CV). NA: number of father genotypes in clutch is unknown, because those metamorphs were clustered without clutch samples.

[a]Clutch samples with more than one father genotype, but not for each father genotype metamorphs were found.

genetic effects concerning growth rate and therefore developmental time.

4.3 | Multiple paternity and differences in metamorphic traits

Multiple paternity is frequently observed in anuran species and was detected in three European explosive breeders (*R. temporaria*: Laurila & Seppä, 1998; *R. arvalis*: Knopp & Merilä, 2009; *R. dalmatina:* Lodé & Lesbarréres, 2004). The advantage of a polyandrous or lek mating system is higher genetic diversity of progeny from a mother, which leads to increased survival probabilities and fitness of offspring (Jennions & Petrie, 2000). This ensures that at least some offspring will survive in unpredictable environments (Yasui, 1998). In our study, the different fathers did not sire an equal number of offspring per clutch. This effect is due to the external fertilization process. The sperm is released simultaneously with the eggs of the female, and fertilization can take place within minutes in large breeding aggregations (Savage, 1961). However, even a few seconds after egg deposition clutch piracy can occur and another "sneaky" male can fertilize the remaining unfertilized eggs (Vieites et al., 2004), which results in different numbers of offspring sired by several males. Therefore, multiple paternity is unlikely an effect of active mate choice of females within the breeding aggregation (Dittrich et al., 2018). The advantage of increased genetic variability among offspring within a clutch will increase the chance that some individuals will survive. Therefore, polygamous mating systems can be seen as a bet-hedging strategy to decrease variability of survivorship between years, especially when the environment is unpredictable and multiple paternity has no additional cost (Yasui, 2001).

We show that multiple paternity leads to higher variability of developmental time in metamorphs within a matriline and on average, longer period of emigration from offspring of the respective female. Additionally, we showed that the effective number of fathers increases this variability.

The effect of higher variability was not detected for size and body condition of offspring, which indicates that these metamorphic traits are probably more influenced by other parameters, such as maternal provisioning or environmental cues. Even when intrinsic effects were found under controlled laboratory conditions, these effects could not be detected under field conditions due to countergradient variation (Laugen et al., 2003). Additionally, it was shown before that those traits, which are important indicators of future fitness, should not be affected by additive genetic variance (Berven & Gill, 1983). Therefore, SVL and BCI seem to be more important proxies for future fitness of metamorphs in our system than developmental time.

4.4 | Influence of number of offspring and number of fathers on metamorphic traits

Due to an inverse relationship between reproductive investment per offspring and number of offspring (Charnov & Ernest, 2006; Smith & Fretwell, 1974), individual females have to trade-off number and size

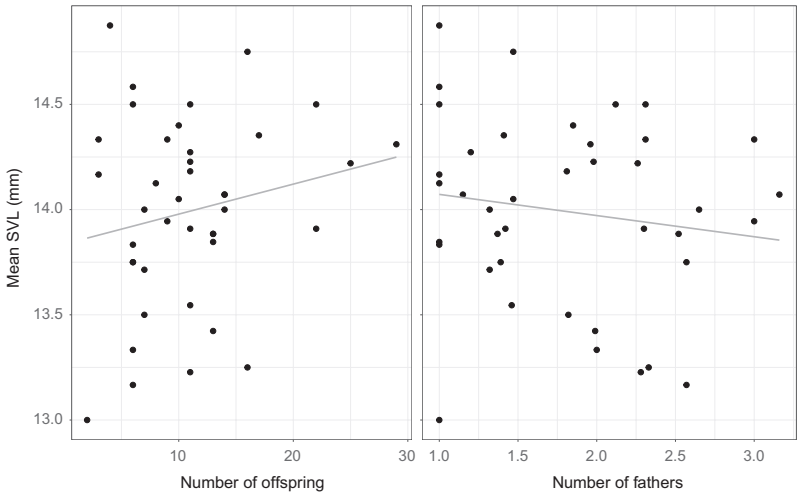

FIGURE 5 Mean snout-vent length (SVL in mm) of metamorphs per matriline (n = 40) in relation to the number of offspring per matriline (left) and number of fathers (right). The gray line is the linear model of number of offspring and number of fathers to mean SVL; $F(3,36) = 2.909$, $p = 0.04771$

of progeny. Therefore, we examined a possible trade-off between the number of successfully metamorphosed offspring per matriline (after natural selection) and their size/body condition and developmental time. We found a positive relationship between the number of metamorphosed offspring and the metamorphic size, but no influence of developmental time, which seems to be in contrast to the known models. Most models of optimal timing and size of metamorphosis are based on maximizing growth rate, to minimize mortality risk in the aquatic and terrestrial habitat and/or taking time constraints into consideration (Rowe & Ludwig, 1991; Rudolf & Rödel, 2007; Wilbur & Collins, 1973). A rapid growth could be favored under time constraints or/and if a minimum size has to be reached for niche transition, or if predation is size dependent (Arendt, 1997). We did not monitor predator densities in the pond, but we observed alpine newts (*Ichthyosaurus alpestris*) sitting under freshly laid clutches, feeding on the embryos. Therefore, we think that predation pressure in the early development could have been high, which could lead to smaller size and lower size variability of progeny (van Buskirk & Relyea, 1998). Additionally, high tadpole densities lead to slower growth and smaller size at metamorphosis (Loman, 2004). In natural populations, densities are high in the beginning (large number of eggs from clutches) and decrease over time due to predation and high mortality in the larval stage (Wilbur, 1980). Therefore, a longer developmental time can be beneficial when tadpoles and predator densities are decreasing over time and single individuals attain more resources for growth in the aquatic stage, which could promote better/higher BCI at metamorphosis. This would be supported by the positive trend we found in BCI over time, where a longer development leads to higher BCI and therefore higher survival probability (Scott et al., 2007). We could not detect any influence of number of fathers or number of successfully developing metamorphs on BCI.

Still, we found a higher number of offspring with bigger body size in single matrilines. In anurans, the egg size correlates strongly with body size (Cummins, 1986) and has a negative relationship with egg number (Jørgensen, 1981). The egg size influences growth and developmental rates, but is not per se responsible for differences in metamorphic size (Loman, 2002). We cannot rule out the possibility of different age/size classes of females and therefore different provisioning or number of eggs per female. However, body size differences have been small in our study and could be canalized, as this trait is highly fitness relevant (Berven & Gill, 1983) in our study pond. The heritability of traits could differ dependent on different selection pressures in the environment, with lower heritability in canalized traits (Berven & Gill, 1983). Therefore, developmental time seems to have a higher heritability because variability in this trait is influenced by additive effects of fathers, while body size and condition were only marginally affected by multiple paternity.

5 | SUMMARY

In our study, we could show that metamorphic traits differ between matrilines in the same environment, which indicates that there are underlying intrinsic genetic effects from the parents. However, SVL and BCI differed only marginally between matrilines, an indication for strong environmental effects that are counteracting the intrinsic growth rates. These environmental effects could be predation pressure and the amount of food resources, as well as temperature and desiccation risk. We show that multiple paternity is very common in this *R. temporaria* population and increases the variability in metamorphic traits, especially in developmental time of offspring from the same matriline. This increase seems to be due to additive genetic effects of multiple fathers on developmental time. These findings suggest that SVL and BCI are more influenced by environmental factors.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

MOR and HF designed the research; JH performed the research; CD and JH analyzed the data; CD, JH, MOR, and HF wrote the manuscript.

DATA ACCESSIBILITY

Data and R-source code to reproduce the analysis and figures are archived at Dryad Digital Repository and are accessible with the following link: https://doi.org/10.5061/dryad.mf4h560.

ORCID

Carolin Dittrich https://orcid.org/0000-0002-4447-4481
Mark-Oliver Rödel https://orcid.org/0000-0002-1666-195X

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

6 Supplementary material

Supplementary material: Paper 1

Temporal migration patterns and mating tactics influence size-assortative mating in *Rana temporaria*.

Supplement S1-S3

Table S1 Summary of different variables per day for each year and location: number of males (N_M_all) and females (N_F_all), SVL of all males (SVL_M_all) and females (SVL_F_all), number of pairs per day (N_pairs), SVL of males and females in the pairs (SVL_M_pair and SVL_F_pair), operational sex ration (OSR), correlation coefficient after Pearson (cor_coef) with respective P-value (P) and lower and upper confidence interval (ci_low, ci_up). Data are separated by location: FS = Fabrikschleichach; KW = Kleiwiesen

day	year	location	N_M_all	SVL_M_all	N_F_all	SVL_F_all	N_pairs	SVL_M_pair	SVL_F_pair	OSR	cor_coef	P	ci_low	ci_up
1	2010	FS	8	67.19	3	76.42	2	NA	NA	2.67	NA	NA	NA	NA
2	2010	FS	19	67.93	15	73.40	14	68.76	73.73	1.27	0.41	0.15	-0.15	0.77
3	2010	FS	32	67.24	26	70.60	20	67.24	70.87	1.23	0.28	0.23	-0.18	0.64
4	2010	FS	8	67.55	8	73.74	6	68.10	73.09	1.00	-0.03	0.96	-0.82	0.80
5	2010	FS	8	67.94	9	69.91	7	68.48	70.58	0.89	0.60	0.16	-0.28	0.93
6	2010	FS	10	67.24	10	69.95	9	68.02	70.09	1.00	0.03	0.94	-0.65	0.68
7	2010	FS	9	65.52	7	67.36	5	64.42	67.77	1.29	-0.02	0.98	-0.89	0.88
8	2010	FS	4	67.65	3	76.60	3	67.01	76.60	1.33	NA	NA	NA	NA
9	2010	FS	5	69.48	6	70.90	4	70.90	71.23	0.83	-0.06	0.94	-0.97	0.96
11	2010	FS	1	68.20	1	60.30	1	68.20	60.30	1.00	NA	NA	NA	NA
12	2010	FS	2	68.20	0	NA	0	NA	NA	NA	NA	NA	NA	NA
13	2010	FS	1	58.22	1	62.38	1	58.22	62.38	1.00	NA	NA	NA	NA
1	2013	FS	63	72.44	27	76.13	NA	NA	NA	2.33	NA	NA	NA	NA
2	2013	FS	82	72.16	65	75.35	53	71.65	76.51	1.26	0.47	0.00	0.22	0.65
3	2013	FS	53	71.44	26	72.12	18	70.44	72.36	2.04	0.45	0.06	-0.02	0.76
4	2013	FS	34	70.46	25	70.72	17	70.82	72.26	1.36	0.15	0.57	-0.36	0.59
5	2013	FS	9	63.06	5	69.00	4	62.63	70.75	1.80	0.97	0.03	0.19	1.00

day	year	location	N_M_all	SVL_M_all	N_F_all	SVL_F_all	n_pairs	SVL_M_pair	SVL_F_pair	OSR	cor_coef	P	ci_low	ci_up
6	2013	FS	1	73.00	2	66.25	1	73.00	75.00	0.50	NA	NA	NA	NA
7	2013	FS	3	66.17	3	67.83	1	72.50	73.00	1.00	NA	NA	NA	NA
8	2013	FS	1	68.50	1	74.00	1	68.50	74.00	1.00	NA	NA	NA	NA
9	2013	FS	1	58.00	1	66.50	1	58.00	66.50	1.00	NA	NA	NA	NA
1	2014	FS	12	71.12	10	71.73	NA	NA	NA	1.20	NA	NA	NA	NA
2	2014	FS	43	71.27	34	77.31	21	71.40	77.50	1.26	0.30	0.18	-0.15	0.65
3	2014	FS	9	67.50	9	69.18	5	66.00	68.50	1.00	0.38	0.52	-0.75	0.95
4	2014	FS	23	71.13	16	75.25	11	70.18	76.05	1.44	0.45	0.17	-0.21	0.83
5	2014	FS	15	70.60	11	73.82	8	72.81	75.25	1.36	-0.18	0.67	-0.79	0.60
6	2014	FS	4	68.25	3	72.33	3	63.83	72.33	1.33	NA	NA	NA	NA
1	2015	FS	3	74.83	0	NA	NA	NA	NA	0.00	NA	NA	NA	NA
2	2015	FS	3	72.33	0	NA	NA	NA	NA	0.00	NA	NA	NA	NA
4	2015	FS	43	71.36	18	75.53	15	71.87	75.70	2.39	0.00	1.00	-0.51	0.51
5	2015	FS	76	70.68	38	77.04	36	69.69	77.21	2.00	0.09	0.60	-0.24	0.41
6	2015	FS	78	71.26	44	76.41	42	71.37	76.50	1.77	0.09	0.58	-0.22	0.38
7	2015	FS	12	69.50	11	71.27	11	69.09	71.27	1.09	0.18	0.60	-0.47	0.70
8	2015	FS	45	69.11	23	72.74	23	70.04	72.74	1.96	0.17	0.43	-0.26	0.55
9	2015	FS	11	70.91	5	70.40	4	68.25	70.50	2.20	0.72	0.28	-0.78	0.99
10	2015	FS	5	67.00	2	68.00	2	67.50	68.00	2.50	NA	NA	NA	NA
11	2015	FS	6	71.33	3	79.67	3	68.67	79.67	2.00	NA	NA	NA	NA
12	2015	FS	99	70.94	52	73.06	47	71.13	73.45	1.90	-0.09	0.57	-0.36	0.21
13	2015	FS	33	70.45	15	74.27	15	61.40	66.20	2.20	0.29	0.30	-0.27	0.70
14	2015	FS	36	69.56	13	75.31	8	68.25	73.13	2.77	-0.30	0.47	-0.83	0.51
15	2015	FS	25	67.88	5	70.00	4	68.50	68.25	5.00	-0.70	0.30	-0.99	0.79
16	2015	FS	42	66.79	14	68.00	12	65.75	68.50	3.00	-0.01	0.97	-0.58	0.57
17	2015	FS	10	67.40	6	67.17	4	63.25	69.25	1.67	0.40	0.60	-0.91	0.98
1	2016	FS	2	67.00	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	2016	FS	0	NA	1	73.50	NA	NA	NA	NA	NA	NA	NA	NA
3	2016	FS	0	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA

day	year	location	N_M_all	SVL_M_all	N_F_all	SVL_F_all	n_pairs	SVL_M_pair	SVL_F_pair	OSR	cor_coef	P	ci_low	ci_up
4	2016	FS	5	66.20	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	2016	FS	26	67.04	12	70.25	11	65.09	70.73	2.17	0.55	0.08	-0.08	0.86
6	2016	FS	31	68.06	14	71.50	12	67.25	72.25	2.21	0.76	0.00	0.32	0.93
7	2016	FS	19	67.58	13	67.62	8	68.00	69.00	1.46	0.33	0.43	-0.49	0.84
8	2016	FS	72	66.19	43	70.81	27	66.52	70.33	1.67	-0.03	0.88	-0.41	0.35
9	2016	FS	40	63.50	24	66.00	15	64.13	66.07	1.67	0.47	0.08	-0.06	0.79
10	2016	FS	2	59.00	4	67.25	2	59.00	68.00	0.50	NA	NA	NA	NA
11	2016	FS	38	62.42	35	63.69	23	61.43	62.96	1.09	0.33	0.13	-0.10	0.65
12	2016	FS	21	62.62	23	65.48	14	63.36	65.57	0.91	0.18	0.54	-0.39	0.65
13	2016	FS	22	61.95	22	66.14	NA	NA	NA	1.00	NA	NA	NA	NA
14	2016	FS	33	62.12	16	64.63	12	64.83	65.67	2.06	0.58	0.05	0.02	0.87
15	2016	FS	10	63.90	4	62.50	3	65.33	63.00	2.50	NA	NA	NA	NA
1	2012	KW	9	65.29	9	65.10	NA	NA	NA	1.00	NA	NA	NA	NA
2	2012	KW	2	67.25	2	63.30	2	67.25	63.30	1.00	NA	NA	NA	NA
3	2012	KW	17	66.39	17	62.82	17	66.39	62.82	1.00	0.07	0.79	-0.42	0.53
5	2012	KW	80	67.91	23	65.96	18	70.83	66.06	3.48	0.38	0.12	-0.11	0.72
6	2012	KW	41	68.78	23	65.87	22	70.59	65.95	1.78	0.02	0.92	-0.40	0.44
7	2012	KW	53	69.64	25	68.28	24	69.38	68.38	2.12	-0.22	0.31	-0.57	0.21
9	2012	KW	40	72.65	30	68.80	30	74.03	68.80	1.33	0.26	0.17	-0.11	0.57
10	2012	KW	51	70.78	15	67.73	15	72.40	67.73	3.40	0.04	0.87	-0.48	0.54
12	2012	KW	35	63.31	0	NA	9	65.29	65.10	0.00	0.15	0.70	-0.57	0.74
1	2013	KW	14	74.77	3	70.87	NA	NA	NA	4.67	NA	NA	NA	NA
2	2013	KW	6	74.02	6	73.42	6	74.02	73.42	1.00	0.46	0.36	-0.56	0.93
3	2013	KW	9	72.82	9	69.09	9	72.82	69.09	1.00	0.34	0.37	-0.42	0.82
4	2013	KW	58	71.51	7	72.86	7	71.73	72.86	8.29	0.07	0.88	-0.72	0.78
5	2013	KW	5	77.10	5	74.64	5	77.10	74.64	1.00	-0.68	0.21	-0.98	0.51
6	2013	KW	1	74.10	1	70.70	1	74.10	70.70	1.00	NA	NA	NA	NA
7	2013	KW	4	69.00	4	70.38	4	69.00	70.38	1.00	0.34	0.66	-0.92	0.98

Table S2. Summary of the linear mixed model output for migration data in Fabrikschleichach, with estimates and standard deviation of fixed effects (day, sex), random effect (year) and model validation parameters.

	Size (SVL)
day	-0.442 ± 0.043
sex male	-3.218 ± 0.275
constant	74.648 ± 1.109
n year	5
standard deviation	2.364
n	2,098
log likelihood	-6,771.854
AIC	13,553.710
BIC	13,581.950
marginal R^2	0.1314061
conditional R^2	0.2456540

Table S3. Summary of the mating speed behavior model (glm with binomial family). Given are estimates with standard error in brackets and model validation parameters.

	Dependent variable:
	win
small_male_SVL	0.228
	(0.187)
large_male_SVL	0.259**
	(0.124)
Female_SVL	0.205
	(0.168)
Constant	-44.211**
	(21.914)
Observations	44
Log Likelihood	-24.153
Akaike Inf. Crit.	56.307
Note:	*p<0.1; **p<0.05; ***p<0.01

Supplementary material: Paper 3

The freedom of choice – Female mate choice behaviour in *Rana temporaria*.

All video and audio files can be found on the accompanying CD-ROM, together with the electronic version of this thesis

Video S1 : attempt of a male *Rana temporaria* to grab a female, first attempt failed, second attempt towards different female was successful

Video S2 : female *R. temporaria* trying to rotate her body out of amplexus

Audio S3: female release call; grunting sound emitted from a female when grabbed by a male

Audio S4: female release call; squeaking sound emitted from a female when grabbed by a male

Video S5: female *R. temporaria* feigning death

Supplementary material: Paper 4

Matriline effects on metamorphic traits in a natural system in the European common frog (*Rana temporaria*).

Supplemental Information for:

Matriline effects on metamorphic traits in a natural system in the European common frog (*Rana temporaria)*

Carolin Dittrich, Juliane Huster, Mark-Oliver Rödel & Heike Feldhaar

Table of content

Figure S1 Picture of the fenced study pond (GPS: N 49° 54' 53.04348 E 10° 32' 52.974456, WGS84)

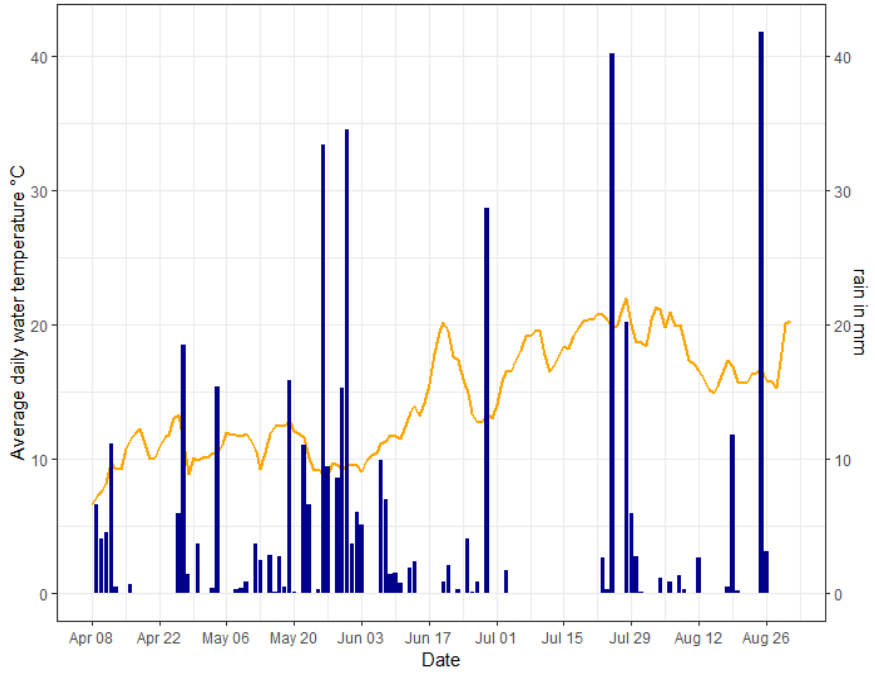


Figure S2 The orange line gives the average daily water temperature (°C) in the pond over the whole period from spawning to last metamorphs emerging (April 08th – August 31st 2013). Temperature was measured with a Thermochron iButton© every 3 hours and mean calculated per day. The blue bars represent the daily amount of rain in mm, measured at a local weather station 2.5 km away from the study pond.

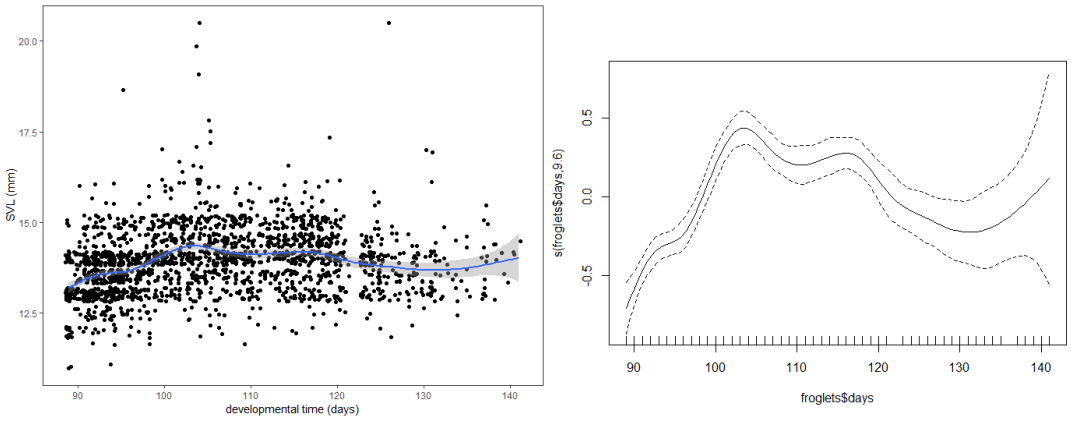
Table S1 PCR mastermix per sample for amplifying microsatellite DNA.

component	company	information	volume [µl]
HPLC-H20	VWR		7.75
10x reaction buffer Y	Peqlab	10 x reaction buffer Y („high yields"): 200 mM Tris-HCL (pH 8.55), 160 mM (NH4)2SO4, 0.1 % Tween 20, 20 mM	1.25
dNTP	Genaxxion	pH 7.5, 2 mM	1
Forward primer	BioScience Metabion	stock solution: 100 pmol/µl, 1:10 diluted	0.2
Reverse primer	Metabion	stock solution: 100 pmol/µl, 1:10 diluted	0.2
Taq	Peqlab	5 U/µl	0.1
+ 2 µl template DNA			

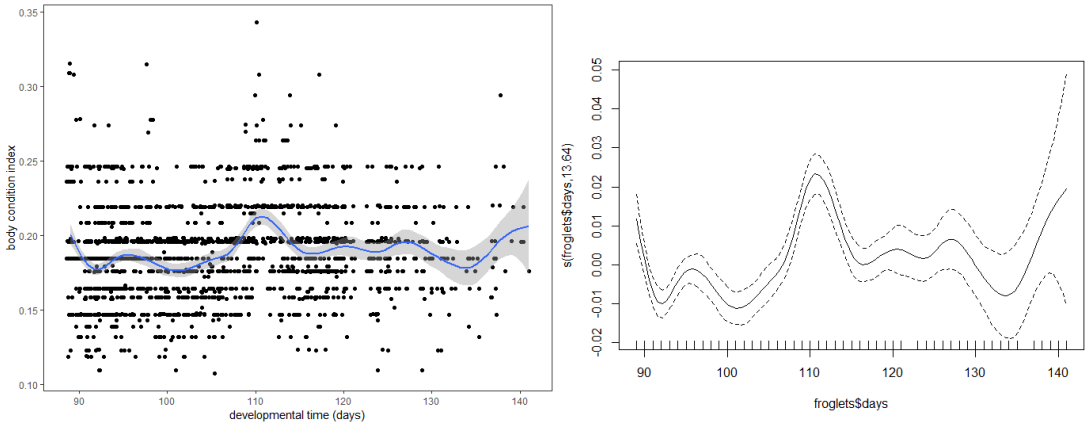
Table S2 Primer sequences and characteristics of seven microsatellite loci in *R. temporaria* (Matsuba & Merilä, 2009). Primers were labelled at 5' end.

locus	core motif	repeats	temp. [°C]	primer sequence (5'-3')	label	allele range [bp]
BFG046	TG	29	55	F-GGAGCATTGGAAAGAACACAAT	-	190-280
				R-GTTTGCGTAGGTAGGCCATGT	IRD700	
BFG090	CTAT	16	55	F-CCTGGGCATTTACCTCATACAT	-	185-320
				R-TCCCTATACGCATTCCAAAATC	IRD700	
BFG099	ACTC	14	55	F-CAGTAAGGAATGGATACTAAGC	-	90-180
				R-TCCAGTGTAGCATAACAGAGT	IRD700	
BFG203	TTCT*2	(23)(4)	55	F-ATATCTTCCCTTTCCCCTTTCA	IRD800	165-234
				R-GCAACAGAGCAGACAGAGAGAG	-	
BFG237	GATA	19	55	F-GGATTCTACGGATCTTTGGACA	IRD800	200-300
				R-CCTTCCATTCTGTTTGTTAGGC	-	
BFG242	GATA	28	55	F-GGACCTCAATGGCCTTATCAGT	IRD700	210-290
				R-CAATCAACTGGCCCCACC	-	
BFG250	GATA	20	55	F-CCTGTTAGAGAAGCCGATCATT	IRD800	220-338
				R-TTGGACTGGAAGTATTGGGAGT	-	

temp. = annealing temperature; primer sequences: F = forward primer, R = reverse primer; IRD-700/800 = fluorescence dye label



Figures S3 Relationship of developmental time in days and SVL in mm of *Rana temporaria* metamorphs (*n* = 1943). Left: Datapoints are jittered to avoid overplotting. Blue line is the GAM (method=REML, cubic regression spline, 15 knots). Deviance explained (R^2) is 12.4% and time has a significant effect on size at metamorphosis ($p < 0.001$), but not a linear one. Right: Model output for the relationship of developmental time and body size.



Figures S4 Relationship of developmental time in days and BCI in g of *Rana temporaria* metamorphs (*n* = 1943). Left: Datapoints are jittered to avoid overplotting. Blue line is the GAM (method=REML, cubic regression spline, 20 knots). Deviance explained (R^2) by the model is 8.5% and time has a significant effect on BCI at metamorphosis ($p < 0.001$), but not a linear one. Right: Model output for the relationship of developmental time and body condition index.

Table S3 Number and effective number of alleles per locus (loci derived from Matsuba & Merilä, 2009), observed and expected heterozygosity.

Locus	Number of alleles	Effective number of alleles (Ae)	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)
BFG046	25	3.6	0.61	0.72
BFG090	23	13.2	0.92	0.92
BFG099	16	5.2	0.83	0.82
BFG203	14	5.2	0.83	0.81
BFG237	15	9.9	0.90	0.90
BFG242	15	9.6	0.79	0.90
BFG250	20	7.8	0.88	0.87

Appendix S1. **Methodological details on sibship analysis**

Clutch sample: From the total number of 228 embryos, 177 were successfully genotyped (five loci: 14, six loci: 49, seven loci: 114). In eleven clutch samples we found two father genotypes. For three of these multiple paternity clutch samples, we detected offspring of only one of these fathers when assigning metamorphs to sibships.

Metamorph samples: For 28 individuals the amplification of microsatellite loci failed completely. The amplification for at least five out of seven loci was successful in 60% of the individuals.

There was no locus indicating large allele dropout and only one locus (Locus BFG242) showed stuttering that could lead to scoring errors. Therefore, the scoring of this locus was checked manually again. Two loci (Locus BFG046 and Locus BFG242) showed more homozygotes than expected and may contain null alleles. However, the presence of related offspring and uneven numbers of offspring per matriline may cause such deviation from HWE (Wigginton, Cutler, & Abecasis, 2005). Hence, the loci were not excluded from further analysis, because the assignment of metamorphs to sibship groups had lower probabilities without the loci deviating from HWE.

Wigginton, J. E., Cutler, D. J., & Abecasis, G. R. (2005). A note on exact tests of Hardy-Weinberg equilibrium. *The American Journal of Human Genetics, 76*, 887-893. https://doi.org/https://doi.org/10.1086/429864

Table S4 Results of a Dunn test with fdr correction for multiple testing for snout-vent length of metamorphs between matrilines. Given are the respective comparisons of matriline number (M1-M40), the z-value, the unadjusted p-value (res.P.unadj) and the adjusted p-value with fdr correction (res.P.adj). Comparisons are sorted from smallest to largest adjusted p-value.

res.Comparison	res.Z	res.P.unadj	res.P.adj
M10 - M17	-4.308555544	1.64E-05	0.012817288
M17 - M33	3.911072452	9.19E-05	0.035836008
M17 - M36	3.665007242	0.000247332	0.064306212
M10 - M40	-3.565988404	0.000362487	0.070685043
M17 - M21	3.489585729	0.00048377	0.075468098
M17 - M25	3.15670079	0.00159565	0.082973793
M17 - M24	3.116851142	0.001827938	0.083870118
M33 - M37	-3.067166659	0.002160983	0.084278351
M10 - M37	-3.409183391	0.000651577	0.08470496
M04 - M10	3.296183959	0.000980078	0.084940108
M12 - M17	-3.165146672	0.001550048	0.086359813
M36 - M40	-3.123278304	0.001788485	0.087188666
M14 - M17	-3.069153682	0.002146661	0.088126096
M12 - M40	-2.909607191	0.003618833	0.088209047
M17 - M32	3.223826856	0.001264899	0.089692806
M15 - M40	-2.909607191	0.003618833	0.0910545
M22 - M33	3.023888974	0.00249548	0.092689258
M21 - M40	-3.069675896	0.002142912	0.09285951
M15 - M17	-3.165146672	0.001550048	0.093002876
M36 - M37	-2.981240932	0.002870828	0.093301921
M23 - M40	-2.909607191	0.003618833	0.09408965
M22 - M36	2.989995439	0.002789816	0.094611156
M17 - M19	3.297965236	0.000973882	0.094953486
M10 - M29	-2.913487248	0.003574164	0.096132696
M04 - M33	2.870236425	0.00410165	0.096948089
M21 - M22	-2.993074548	0.002761823	0.097919172
M33 - M40	-3.223971672	0.001264259	0.098612206
M12 - M22	-2.91593965	0.003546191	0.098786746
M17 - M23	3.165146672	0.001550048	0.100753115
M21 - M37	-2.943599922	0.003244191	0.101218753
M15 - M22	-2.91593965	0.003546191	0.102445514
M19 - M40	-2.842296351	0.004478984	0.102753153
M10 - M22	-3.310713737	0.000930584	0.103693594
M12 - M37	-2.811049068	0.004938025	0.104098915
M22 - M23	2.91593965	0.003546191	0.106385726
M15 - M37	-2.811049068	0.004938025	0.106990552
M23 - M37	-2.811049068	0.004938025	0.110047425
M19 - M22	-2.767775798	0.005644027	0.115851077
M07 - M10	2.738616043	0.006169838	0.123396765
M04 - M36	2.721261783	0.006503324	0.126814815
M19 - M37	-2.707340084	0.006782474	0.129032429

M16 - M17	-2.682299257	0.007311802	0.135790608
M04 - M21	2.655308281	0.007923596	0.13734233
M03 - M17	-2.669322947	0.007600433	0.137868324
M09 - M17	-2.644418985	0.008183129	0.13875741
M10 - M31	-2.658983366	0.007837683	0.138940749
M25 - M40	-2.635358501	0.008404846	0.139484679
M17 - M18	2.601011338	0.009294938	0.145001036
M04 - M12	2.514947417	0.011905014	0.145092356
M12 - M29	-2.536849647	0.011185498	0.145411477
M08 - M17	-2.584957608	0.009739097	0.146086456
M10 - M35	-2.603947985	0.009215674	0.146698487
M21 - M29	-2.575494514	0.010009686	0.147312362
M04 - M15	2.514947417	0.011905014	0.147395409
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M22 - M25	2.554408871	0.010636827	0.148155804
M29 - M33	2.586456702	0.009696835	0.148304539
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M17 - M39	2.605144498	0.009183552	0.149232721
M32 - M40	-2.545317272	0.010917849	0.149402142
M04 - M23	2.514947417	0.011905014	0.149772754
M29 - M36	2.556654229	0.010568421	0.149879425
M23 - M29	-2.536849647	0.011185498	0.150425666
M24 - M40	-2.515782506	0.011876845	0.151867854
M25 - M37	-2.487521317	0.012863674	0.154364093
M14 - M40	-2.433623828	0.014948521	0.176664344
M22 - M24	2.420848478	0.01548433	0.177614374
M22 - M32	2.42345824	0.015373521	0.178975316
M17 - M28	2.24991653	0.024454244	0.190743107
M04 - M19	2.389445453	0.016873829	0.190747636
M16 - M40	-2.251498201	0.024354001	0.191880006
M09 - M40	-2.254943204	0.024136894	0.192109973
M16 - M22	-2.238615485	0.025180946	0.192560174
M09 - M22	-2.255091939	0.024127559	0.194015419
M17 - M26	2.238985055	0.025156889	0.194280922
M32 - M37	-2.376756948	0.017465592	0.1946166
M07 - M12	2.256628668	0.024031287	0.195254211
M12 - M35	-2.204899211	0.027461168	0.19651111
M31 - M33	2.304268935	0.021207552	0.196927265
M12 - M20	-2.351387188	0.01870356	0.197145634
M07 - M15	2.256628668	0.024031287	0.197309518
M10 - M34	-2.307629925	0.021019729	0.197534799
M12 - M31	-2.312029907	0.020776035	0.197625701
M15 - M35	-2.204899211	0.027461168	0.198330657
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M15 - M20	-2.351387188	0.01870356	0.199846259
M15 - M31	-2.312029907	0.020776035	0.200065525
M21 - M35	-2.208650717	0.027198945	0.200143179

M23 - M35	-2.204899211	0.027461168	0.200184215
M07 - M33	2.335195115	0.01953323	0.200472626
M01 - M17	-2.211249149	0.027018589	0.200709515
M10 - M27	-2.192879243	0.028316081	0.200786753
M20 - M33	2.257485011	0.023977785	0.201104004
M14 - M22	-2.337958755	0.019389388	0.201649634
M17 - M34	2.212106504	0.026959307	0.202194801
M18 - M40	-2.259089605	0.023877812	0.202442316
M23 - M31	-2.312029907	0.020776035	0.202566344
M20 - M23	2.351387188	0.01870356	0.202621902
M19 - M29	-2.31653516	0.020529067	0.202692054
M35 - M36	2.177551066	0.029439476	0.203210542
M24 - M37	-2.355430566	0.018501254	0.203253216
M18 - M22	-2.274566596	0.022931942	0.203260394
M20 - M36	2.265895085	0.023457808	0.203300999
M07 - M21	2.287686666	0.022155775	0.203311816
M33 - M35	-2.21307172	0.026892701	0.203653467
M31 - M36	2.28228741	0.022472377	0.203819237
M22 - M39	2.179622844	0.02928543	0.203952102
M39 - M40	-2.183052656	0.029031933	0.204008175
M20 - M21	2.323210773	0.020167832	0.204297522
M05 - M17	-2.259251957	0.023867717	0.204580428
M14 - M37	-2.267469177	0.02336158	0.204741935
M07 - M36	2.276066793	0.022842012	0.204790454
M21 - M31	-2.317161168	0.020494954	0.20494954
M03 - M40	-2.155500797	0.031122666	0.212944559
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M18 - M37	-2.13301363	0.032923608	0.221382882
M03 - M22	-2.127839521	0.033350391	0.222335943
M09 - M37	-2.119986936	0.034007147	0.224793006
M22 - M26	2.103642176	0.035409672	0.226389706
M10 - M28	-2.106017373	0.035202843	0.22692742
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M16 - M37	-2.109460829	0.034904824	0.22878792
M10 - M30	-2.091337162	0.036497851	0.231449785
M12 - M27	-2.07169918	0.038293504	0.235188449
M15 - M27	-2.07169918	0.038293504	0.237055024
M23 - M27	-2.07169918	0.038293504	0.238951464
M25 - M29	-2.061857724	0.039221283	0.239004696
M10 - M13	-2.074323244	0.038049301	0.239342374
M19 - M20	-2.053447615	0.040029185	0.242036935
M12 - M34	-2.022819785	0.043091731	0.247143751
M37 - M39	2.041015283	0.041249308	0.247495848
M26 - M40	-2.032225641	0.042130818	0.248954835
M15 - M34	-2.022819785	0.043091731	0.248974446
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M08 - M40	-1.984320542	0.047220118	0.259378111
M21 - M27	-1.987120541	0.04690904	0.25949682
M12 - M30	-1.963133947	0.049630612	0.259811258
M03 - M37	-1.998695784	0.045641279	0.25985546
M05 - M40	-1.947325647	0.051495706	0.260822406
M07 - M19	1.990935344	0.046487996	0.260867891
M13 - M15	1.952676574	0.050857931	0.260981489
M04 - M24	1.987573672	0.046858861	0.261070794
M15 - M30	-1.963133947	0.049630612	0.261566739
M08 - M22	-1.95599604	0.050465621	0.262421227
M06 - M12	1.967148974	0.049166039	0.262667878
M13 - M23	1.952676574	0.050857931	0.262709843
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M06 - M15	1.967148974	0.049166039	0.26447938
M21 - M34	-1.972093187	0.04859897	0.265085289
M12 - M28	-1.928397398	0.053805715	0.265623151
M06 - M23	1.967148974	0.049166039	0.266316043
M04 - M14	1.882480503	0.059770805	0.266407015
M15 - M28	-1.928397398	0.053805715	0.267315018
M01 - M22	-1.878449887	0.060319646	0.267325706
M26 - M37	-1.933667518	0.05315402	0.267484746
M11 - M12	1.882834485	0.059722802	0.267722906
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M27 - M33	1.865848697	0.062062537	0.271959432
M34 - M36	1.907003257	0.056520166	0.272134131
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M08 - M23	1.892440369	0.058432332	0.272917477
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M33 - M34	-1.910146833	0.05611431	0.273557263
M17 - M30	1.896276113	0.057923543	0.273820386
M17 - M38	1.896538947	0.057888815	0.275324852
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M13 - M21	1.85323801	0.063848252	0.276675758
M21 - M28	-1.838608991	0.065972718	0.28430232
M01 - M40	-1.833620135	0.066710401	0.28590172

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M08 - M37	-1.811683904	0.070035052	0.29369538
M06 - M21	1.81587653	0.069389314	0.294150354
M07 - M17	-1.812863466	0.069852882	0.294514855
M12 - M38	-1.798009063	0.072175572	0.294748408
M15 - M38	-1.798009063	0.072175572	0.296299715
M08 - M21	1.804986877	0.071076736	0.296469806
M14 - M29	-1.791744734	0.073173865	0.297268828
M22 - M28	1.786184295	0.074069423	0.297804897
M23 - M38	-1.798009063	0.072175572	0.297867438
M18 - M29	-1.788408487	0.073710128	0.297895854
M17 - M35	1.798763982	0.072056023	0.29895584
M20 - M25	1.781422734	0.074843417	0.299373668
M30 - M36	1.77155602	0.076468286	0.304312566
M10 - M38	-1.768880337	0.076913846	0.30453198
M28 - M40	-1.759081212	0.078563716	0.309493425
M13 - M36	1.756247995	0.079046073	0.309828828
M25 - M31	-1.745926498	0.080823725	0.31209161
M28 - M36	1.74796694	0.080469752	0.31227068
M09 - M29	-1.748571974	0.080365034	0.313423634
M30 - M33	1.736622732	0.082453784	0.31526447
M11 - M21	1.738822878	0.082065923	0.315327192
M04 - M18	1.731760308	0.083316248	0.317008163
M22 - M34	1.729423804	0.083733273	0.317048314
M16 - M29	-1.716585901	0.08605486	0.319632339
M28 - M33	1.722540066	0.084971731	0.320183336
M13 - M33	1.719608813	0.085503573	0.320638399
M08 - M36	1.716883044	0.086000544	0.320958966
M04 - M09	1.705199743	0.088157178	0.325889094
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M07 - M25	1.68852158	0.09131115	0.328215194
M08 - M33	1.699164523	0.089288189	0.328513147
M04 - M16	1.685555991	0.091881352	0.328749791
M01 - M37	-1.691582763	0.090725555	0.329143875
M19 - M27	-1.689326397	0.091156897	0.329177685
M26 - M29	-1.675278069	0.093879686	0.334366006
M19 - M34	-1.649158397	0.099115189	0.349818313
M29 - M39	1.650630123	0.098814124	0.350340985
M21 - M38	-1.640926884	0.100812594	0.35420641
M22 - M38	1.637822008	0.101458829	0.354878416
M06 - M33	1.631409635	0.102803913	0.357977912
M11 - M36	1.61849649	0.105555643	0.365926228
M03 - M12	1.594662981	0.11078762	0.366162474
M03 - M15	1.594662981	0.11078762	0.367720612
M22 - M30	1.589366373	0.111977702	0.368534209
M01 - M12	1.607776022	0.107884242	0.369077669

M03 - M23	1.594662981	0.11078762	0.369292068
M13 - M22	-1.602866782	0.108964069	0.36953032
M04 - M39	1.604240247	0.108661106	0.370112066
M28 - M37	-1.595641055	0.110568956	0.370145002
M01 - M15	1.607776022	0.107884242	0.370703562
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M11 - M17	-1.574979667	0.115261138	0.377746587
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M03 - M29	-1.564803136	0.117629076	0.379135039
M04 - M26	1.568649498	0.116729632	0.379371305
M18 - M20	-1.565836891	0.117386807	0.379924107
M38 - M40	-1.554434358	0.120080843	0.38386499
M12 - M14	-1.539034082	0.123795976	0.384704627
M24 - M31	-1.548958633	0.121391665	0.3849004
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M19 - M30	-1.549579604	0.121242451	0.385996376
M13 - M19	1.535077838	0.124764709	0.386176481
M14 - M15	1.539034082	0.123795976	0.386243445
M34 - M37	-1.530290971	0.125944722	0.386759383
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M19 - M28	-1.508776465	0.131355917	0.394067751
M12 - M32	-1.500369642	0.133518679	0.394487005
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M23 - M32	-1.500369642	0.133518679	0.397498357
M36 - M38	-1.511775854	0.130590894	0.39789413
M04 - M17	-1.485009348	0.137541348	0.400306908
M05 - M29	-1.485683092	0.137362965	0.401285066
M18 - M31	-1.487499648	0.136882896	0.401385936
M07 - M24	1.47997144	0.138880869	0.402702891
M14 - M20	-1.487636454	0.136846794	0.402794338
M01 - M10	1.474172578	0.140435131	0.40570149
M07 - M32	1.470121111	0.141528956	0.407352714
M08 - M19	1.463948961	0.143207894	0.409165412
M16 - M20	-1.464921363	0.142942374	0.409908277
M22 - M35	1.457101986	0.14508823	0.413024889
M10 - M14	-1.45063575	0.146881315	0.416608821
M17 - M27	1.443762335	0.14880584	0.419020056

M33 - M38	-1.441849791	0.149344753	0.419024846
M07 - M22	-1.445333165	0.148364327	0.419290488
M11 - M19	1.43361065	0.151683403	0.424061126
M14 - M31	-1.429256676	0.152930473	0.424504516
M09 - M31	-1.430137787	0.152677477	0.425315828
M26 - M31	-1.422202607	0.154967442	0.428633351
M12 - M24	-1.411166072	0.158195652	0.429939402
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M23 - M24	-1.411166072	0.158195652	0.43295652
M37 - M38	1.412396986	0.157833104	0.433485284
M01 - M21	1.400299006	0.161423798	0.435676687
M20 - M39	1.402034996	0.160904797	0.435783824
M07 - M18	1.391549803	0.164058768	0.438239176
M10 - M32	-1.392494434	0.163772726	0.438978441
M03 - M21	1.394101755	0.163286879	0.4391854
M16 - M31	-1.384216592	0.166292144	0.442688985
M25 - M27	-1.379203469	0.167832029	0.443759262
M12 - M39	-1.369677894	0.170787472	0.444047428
M04 - M05	1.376549549	0.168651558	0.444419647
M24 - M35	-1.380126938	0.167547565	0.444513947
M15 - M39	-1.369677894	0.170787472	0.445532537
M13 - M37	-1.372095753	0.170033631	0.446552971
M35 - M40	-1.364304267	0.172471823	0.446936951
M23 - M39	-1.369677894	0.170787472	0.447027612
M32 - M35	-1.359928344	0.1738526	0.449023273
M07 - M40	-1.3545283	0.175567891	0.450470246
M30 - M37	-1.356121853	0.175060401	0.450650537
M07 - M14	1.348238614	0.177581649	0.454143233
M08 - M29	-1.338903234	0.180602177	0.460358489
M12 - M16	-1.323040116	0.185822038	0.463070892
M19 - M38	-1.320282719	0.186740643	0.46387803
M15 - M16	-1.323040116	0.185822038	0.464555094
M07 - M26	1.326025003	0.184831416	0.465059691
M14 - M21	1.327793948	0.184246187	0.465087462
M07 - M09	1.330807395	0.183252394	0.465592402
M18 - M35	-1.328661787	0.183959577	0.465871657
M16 - M23	1.323040116	0.185822038	0.46604884
M31 - M39	1.313706899	0.188944858	0.466382878
M01 - M29	-1.313908521	0.188876991	0.467695406
M25 - M34	-1.305919703	0.191579865	0.471395252
M07 - M16	1.282084543	0.19981299	0.476618142
M17 - M31	1.282453375	0.199683651	0.477770698
M26 - M35	-1.283840451	0.199197787	0.478074688
M21 - M32	-1.278058751	0.201228712	0.478531692
M22 - M27	1.291628352	0.196485881	0.478934334
M05 - M12	1.284401989	0.199001338	0.479077294

M03 - M20	-1.292113131	0.196317969	0.480025128
M05 - M20	-1.274539244	0.202472374	0.480025689
M11 - M40	-1.293819871	0.195727647	0.480086682
M04 - M08	1.288418422	0.197600349	0.480150382
M05 - M15	1.284401989	0.199001338	0.480560505
M05 - M23	1.284401989	0.199001338	0.482052929
M09 - M35	-1.261535292	0.207116053	0.48806804
M06 - M25	1.262975347	0.20659804	0.488322639
M14 - M35	-1.24571959	0.212867359	0.500110061
M01 - M36	1.242745708	0.213961555	0.501171209
M03 - M36	1.23587125	0.216506425	0.505613808
M26 - M27	-1.210127601	0.226229933	0.518998083
M16 - M35	-1.207523716	0.227230517	0.51976482
M09 - M12	1.210188272	0.226206657	0.520475495
M03 - M31	-1.196130056	0.23164581	0.520702398
M07 - M39	1.204044615	0.228572335	0.521305326
M06 - M26	1.196535927	0.231487487	0.521850405
M09 - M15	1.210188272	0.226206657	0.522015363
M10 - M24	-1.212919569	0.225160573	0.522694187
M04 - M22	-1.191897478	0.233301447	0.522917037
M13 - M25	1.197005216	0.231304522	0.522949354
M18 - M27	-1.200034514	0.230125936	0.523318456
M35 - M37	-1.198149616	0.230858776	0.523458852
M09 - M23	1.210188272	0.226206657	0.52356437
M01 - M04	-1.189101975	0.234399541	0.523872901
M25 - M30	-1.212979115	0.225137805	0.524201457
M07 - M37	-1.183820624	0.236484076	0.527021656
M01 - M33	1.147142032	0.251322925	0.535606233
M02 - M12	1.148762104	0.250654089	0.535644356
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M12 - M25	-1.152701523	0.249032916	0.538076661
M06 - M18	1.150667425	0.249869083	0.538391946
M03 - M33	1.142243412	0.253352856	0.53846111
M02 - M23	1.148762104	0.250654089	0.538595564
M05 - M31	-1.168399063	0.242645833	0.539212961
M15 - M25	-1.152701523	0.249032916	0.539571318
M22 - M31	1.138943625	0.254726669	0.539909787
M23 - M25	-1.152701523	0.249032916	0.541074302
M11 - M37	-1.158863603	0.246511787	0.541631532
M21 - M24	-1.156559852	0.247452233	0.542170623
M27 - M40	-1.152767079	0.249006	0.54252704
M25 - M28	-1.154417233	0.248329153	0.542567898
M24 - M27	-1.158894909	0.246499024	0.543133443
M14 - M36	1.160425281	0.245875699	0.543294745
M28 - M29	-1.160464805	0.245859615	0.544802557
M35 - M39	1.131364544	0.257901688	0.54515804
M09 - M27	-1.121363212	0.262133289	0.552605311

M27 - M32	1.118275456	0.263449366	0.553882764
M11 - M25	1.111922293	0.266171563	0.558101664
M10 - M39	-1.105312543	0.269024185	0.562570681
M32 - M36	1.102969451	0.270040432	0.563185927
M21 - M39	-1.099126049	0.271713089	0.565163225
M26 - M34	-1.096658168	0.27279085	0.565895911
M08 - M25	1.093115174	0.274343234	0.567606692
M18 - M34	-1.083518796	0.278578213	0.573327192
M01 - M20	-1.080983545	0.279704433	0.574130153
M29 - M34	1.083758853	0.278471733	0.574624212
M06 - M09	1.072491377	0.283499385	0.577361672
M12 - M18	-1.058763801	0.289707363	0.577932847
M26 - M30	-1.073306647	0.283133554	0.578126105
M03 - M07	-1.074895628	0.28242146	0.578185666
M15 - M18	-1.058763801	0.289707363	0.579414726
M06 - M24	1.067430188	0.285777623	0.580485796
M24 - M34	-1.05405119	0.291859481	0.580740804
M18 - M23	1.058763801	0.289707363	0.580904224
M13 - M26	1.062849275	0.28785031	0.581666429
M08 - M20	-1.051000474	0.293258372	0.582039518
M01 - M19	1.063772539	0.287431754	0.582329268
M16 - M27	-1.058763801	0.289707363	0.5824014
M05 - M07	-1.046881779	0.295154107	0.582835957
M14 - M33	1.059759485	0.289254033	0.582992624
M29 - M38	1.044878375	0.296079186	0.583186275
M04 - M40	-1.047819544	0.294721756	0.583459314
M11 - M26	1.042026719	0.297399292	0.584310951
M16 - M21	1.040133408	0.298277924	0.584564776
M03 - M19	1.036277606	0.300072651	0.585141669
M18 - M30	-1.0370769	0.29970002	0.585879738
M14 - M27	-1.032157201	0.301998481	0.585967202
M10 - M16	-1.033061609	0.301575068	0.586604871
M04 - M28	1.022047693	0.306758328	0.590793817
M13 - M18	1.02357649	0.306035354	0.590860337
M06 - M32	1.024399121	0.305646797	0.591574446
M27 - M37	-1.010729707	0.312145815	0.595290307
M32 - M34	-1.011720352	0.311671782	0.595843112
M06 - M16	1.011863271	0.311603433	0.597176112
M26 - M28	-1.013261907	0.310935077	0.597362956
M02 - M22	-1.000271305	0.31717923	0.603414145
M03 - M35	-0.991962149	0.32121599	0.603731259
M05 - M35	-0.990204506	0.32207417	0.60388907
M05 - M21	0.994857165	0.319805739	0.603991468
M27 - M39	0.992808024	0.320803519	0.604412427
M32 - M33	0.995011342	0.319730748	0.605315494
M09 - M34	-0.995612147	0.319438631	0.606233898
M11 - M18	0.981448996	0.326371375	0.610478831

M31 - M40	-0.975860058	0.329133818	0.611248519
M13 - M29	-0.976930343	0.328603639	0.611720378
M25 - M38	-0.97753619	0.32830377	0.61262426
M08 - M26	0.957934784	0.338095645	0.614719354
M06 - M17	-0.949783583	0.342222229	0.615053776
M14 - M19	0.952222878	0.340983961	0.615665484
M01 - M31	-0.953736365	0.340217108	0.615706135
M06 - M22	-0.955172047	0.339490699	0.615820338
M24 - M30	-0.967465365	0.333311447	0.616073291
M06 - M39	0.958010604	0.338057412	0.616085937
M05 - M10	0.959417715	0.337348354	0.616233528
M13 - M24	0.950036412	0.342093751	0.616242785
M02 - M17	-0.960888366	0.336608301	0.616325058
M18 - M28	-0.968452648	0.332818357	0.616623083
M09 - M30	-0.946306143	0.343992461	0.616814069
M06 - M14	0.960959074	0.336572746	0.617709981
M24 - M36	0.96406395	0.335013865	0.617756063
M29 - M30	0.961622317	0.336239357	0.618553534
M26 - M38	-0.936437485	0.349047986	0.624443644
M09 - M13	-0.931804377	0.351437632	0.627279985
M16 - M34	-0.924295272	0.355332604	0.631342667
M04 - M34	0.925577828	0.35466542	0.631595954
M08 - M31	-0.921071404	0.357013149	0.632886945
M30 - M32	0.917710667	0.358770362	0.634559824
M05 - M06	-0.89481777	0.37088451	0.63860909
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M09 - M11	-0.8898273	0.373558625	0.638981859
M02 - M21	0.892715251	0.372009681	0.639135576
M05 - M27	-0.897309119	0.369553992	0.639139941
M24 - M28	-0.890955169	0.372953221	0.639348379
M12 - M33	-0.905864287	0.365007689	0.639788758
M19 - M32	-0.89506657	0.370751503	0.639792417
M13 - M32	0.89931271	0.368486119	0.640131789
M14 - M34	-0.901740805	0.367194571	0.640742205
M09 - M21	0.899885654	0.368181105	0.641029603
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M08 - M18	0.902137285	0.366983945	0.641810486
M23 - M33	-0.905864287	0.365007689	0.642677195
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M16 - M30	-0.873485202	0.382398669	0.651246641
M09 - M28	-0.871133966	0.38368101	0.652006945
M04 - M37	-0.869093104	0.384796208	0.652480527
M02 - M40	-0.866133068	0.386417193	0.653807831
M04 - M38	0.857419334	0.391213178	0.659063237
M18 - M38	-0.853840858	0.393193173	0.659549838
M13 - M16	0.858177177	0.390794637	0.659783153

M09 - M10	0.854701456	0.392716445	0.660169885
M34 - M39	0.847140513	0.396916806	0.664367186
M03 - M27	-0.844195131	0.398560375	0.665689706
M16 - M36	0.840068328	0.400870077	0.668116795
M28 - M32	0.836662693	0.402782195	0.668447047
M24 - M33	0.837789805	0.402148765	0.668818842
M21 - M25	-0.822234615	0.410943382	0.669177114
M11 - M16	0.817522767	0.413629744	0.669359337
M20 - M34	0.818778492	0.412912804	0.669588331
M31 - M37	-0.823174471	0.410408784	0.669704711
M03 - M06	-0.823721572	0.410097779	0.670600142
M11 - M29	-0.819177671	0.412685052	0.67061321
M20 - M38	0.824473365	0.409670644	0.671309038
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M17 - M20	0.830028347	0.406522757	0.671796081
M14 - M30	-0.824951146	0.409399327	0.67227679
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M11 - M32	0.827918999	0.407716374	0.672344127
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M13 - M14	0.806838446	0.419759566	0.676472028
M15 - M19	-0.799126697	0.424216951	0.676665075
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M02 - M37	-0.792214476	0.428235629	0.677533044
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M33 - M39	-0.778281997	0.436402786	0.686278575
M04 - M13	0.773175323	0.439418629	0.686866794
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M01 - M35	-0.742551928	0.457753002	0.695998716
M11 - M14	0.751160396	0.452556131	0.696240202
M07 - M29	-0.749007286	0.453852814	0.69686062
M19 - M39	-0.742721088	0.457650559	0.697202024
M02 - M36	0.751484879	0.452360898	0.697315218
M01 - M26	0.746204444	0.455543924	0.698083027
M08 - M32	0.752020216	0.452038902	0.6981987
M13 - M20	-0.743017751	0.457470933	0.698292227
M07 - M08	0.756901873	0.449108653	0.699211077
M28 - M31	-0.743444925	0.457212354	0.699265953

M09 - M38	-0.752319534	0.451858923	0.699305477
M04 - M30	0.753559683	0.451113661	0.699540071
M11 - M39	0.7546364	0.450467177	0.69992908
M14 - M28	-0.735258401	0.462182137	0.700004014
M20 - M30	0.728515985	0.466297787	0.700794362
M24 - M38	-0.726152838	0.46774508	0.70161762
M12 - M36	-0.729022105	0.465988141	0.701680985
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M05 - M30	-0.717976315	0.472771891	0.706440756
M18 - M21	0.718507953	0.472444146	0.707306015
M08 - M16	0.712809345	0.475963742	0.708495647
M22 - M29	0.703254379	0.481897236	0.709207253
M12 - M26	-0.703470248	0.481762742	0.7103496
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M23 - M26	-0.703470248	0.481762742	0.713045424
M16 - M33	0.706031063	0.48016882	0.713393676
M05 - M13	-0.704475906	0.48113645	0.713472302
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M10 - M12	0.685380338	0.493103948	0.716240371
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M01 - M06	-0.683078566	0.494557198	0.717016012
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M16 - M38	-0.671707526	0.501769909	0.720774455
M02 - M33	0.662934014	0.507372816	0.720857553
M05 - M19	0.661469734	0.508311118	0.720877586
M08 - M35	-0.669876964	0.502936226	0.721121795
M03 - M34	-0.668397328	0.50388	0.721149358
M32 - M38	-0.665498843	0.505731483	0.721152755
M05 - M33	0.672592952	0.501206287	0.72129318
M02 - M19	0.674011981	0.500303697	0.721325108
M06 - M37	-0.66385038	0.506786078	0.721337848
M31 - M38	0.666131041	0.505327345	0.721896208
M17 - M29	0.674245132	0.500155481	0.722446806
M27 - M29	-0.6578221	0.510652452	0.722883689
M01 - M27	-0.656086421	0.511768521	0.723151171
M31 - M34	0.648431482	0.516705914	0.728807618
M08 - M14	0.643226779	0.520076959	0.732238318
M03 - M25	0.639719003	0.522355299	0.73412096
M05 - M28	-0.633777132	0.526226279	0.735585121
M08 - M39	0.633977112	0.526095759	0.736722966
M10 - M18	-0.634238478	0.5259252	0.737808734

M03 - M30	-0.628483853	0.529687021	0.739098169
M20 - M40	-0.62431943	0.532417826	0.741581972
M11 - M20	-0.619836965	0.535365129	0.744357934
M14 - M26	0.617975322	0.536591606	0.744735681
M02 - M29	-0.61510429	0.538485849	0.746037233
M01 - M18	0.613212979	0.539735524	0.746442745
M03 - M13	-0.611469934	0.540888517	0.746713351
M38 - M39	0.605751749	0.544679615	0.750618551
M25 - M36	0.591307756	0.554314234	0.762548681
M14 - M38	-0.585223112	0.558397706	0.762784958
M03 - M11	-0.589284075	0.555670726	0.763068954
M04 - M11	0.585524945	0.558194799	0.763845514
M06 - M08	0.586880399	0.557284035	0.763939451
M26 - M32	-0.574208025	0.56582704	0.770235761
M02 - M26	0.574381052	0.565709972	0.771422689
M13 - M31	-0.568187508	0.569907653	0.774438971
M05 - M38	-0.551115463	0.581554526	0.783441331
M07 - M28	0.554662919	0.579125244	0.784232101
M30 - M31	-0.551652951	0.581186151	0.78429965
M03 - M18	0.548635738	0.583255455	0.784378025
M08 - M27	-0.552655441	0.580499371	0.784730519
M05 - M08	-0.554887442	0.578971652	0.785387633
M09 - M33	0.545686636	0.58528136	0.785747781
M09 - M19	0.54378335	0.586590568	0.786152307
M20 - M35	0.532213143	0.594578381	0.795490802
M14 - M25	0.528263428	0.597316511	0.797785751
M03 - M28	-0.524545069	0.599899482	0.799865976
M02 - M20	-0.511546552	0.6089684	0.810572273
M26 - M39	-0.508106315	0.611378787	0.812394299
M07 - M20	-0.501855514	0.61576916	0.814067703
M24 - M26	0.502989887	0.614971382	0.814393342
M21 - M33	-0.498052302	0.618447179	0.814845945
M04 - M35	0.50326781	0.614775995	0.815519177
M01 - M09	0.495809354	0.620028925	0.81555238
M18 - M36	0.498332573	0.618249653	0.815964009
M20 - M37	-0.489363162	0.624584611	0.820161611
M07 - M38	0.48730948	0.626039031	0.820689822
M28 - M35	-0.483079392	0.629039366	0.823239439
M06 - M28	0.481135422	0.630420254	0.823664654
M12 - M21	-0.472104515	0.63685219	0.826530297
M29 - M40	-0.470112824	0.638274412	0.827000069
M15 - M21	-0.472104515	0.63685219	0.827907847
M21 - M23	0.472104515	0.63685219	0.829289997
M06 - M38	0.463416372	0.643065946	0.8304494
M04 - M07	0.46487964	0.642017652	0.830470594
M05 - M26	0.472104515	0.63685219	0.83067677
M16 - M26	0.461468538	0.644462489	0.830877259

M25 - M32	-0.459453727	0.645908374	0.831367214
M29 - M31	0.450890927	0.652068163	0.832427442
M20 - M27	0.455429687	0.648800142	0.833713526
M02 - M04	-0.450936559	0.652035273	0.833750021
M14 - M18	0.447805018	0.654293922	0.833904018
M01 - M34	-0.453245164	0.650372214	0.834359091
M02 - M25	0.451546182	0.651595948	0.834556387
M11 - M31	-0.442963031	0.657792475	0.835632134
M01 - M30	-0.443004975	0.657762136	0.836956715
M07 - M34	0.439835534	0.660056229	0.837144486
M02 - M18	0.438290921	0.661175406	0.837202625
M04 - M29	-0.430793242	0.666618714	0.837298867
M01 - M13	-0.42769695	0.668871771	0.837431752
M19 - M25	-0.436554188	0.662434693	0.837437699
M35 - M38	0.431902674	0.665812152	0.837634642
M25 - M33	0.428751872	0.668103807	0.837815063
M22 - M37	0.433043564	0.664983122	0.83794319
M01 - M24	0.425367991	0.670568436	0.838210545
M01 - M11	-0.433584949	0.664589866	0.838802744
M03 - M09	0.419685244	0.674715406	0.839358879
M03 - M08	-0.422097565	0.672953805	0.839846349
M03 - M38	-0.420196225	0.674342112	0.840234581
M27 - M28	0.412131043	0.680243377	0.84488827
M06 - M34	0.404849465	0.685588167	0.850172925
M01 - M16	0.40267738	0.687185576	0.85080119
M02 - M31	-0.396809126	0.691508221	0.854796216
M27 - M38	0.387056275	0.698714547	0.858263538
M18 - M32	-0.38948436	0.696917878	0.858761366
M19 - M21	0.387201796	0.698606821	0.859484732
M06 - M29	-0.389723248	0.696741203	0.859902118
M04 - M27	0.38022431	0.703778915	0.863125085
M34 - M35	-0.368648894	0.712389446	0.868224638
M10 - M33	-0.365631266	0.714640241	0.868254499
M09 - M26	0.369380506	0.711844126	0.868917713
M06 - M13	0.372268158	0.709693204	0.869012087
M07 - M13	0.365802327	0.714512583	0.86945369
M18 - M19	0.370172087	0.711254273	0.869558516
M06 - M30	0.360056578	0.718804824	0.870602116
M02 - M09	0.360058517	0.718803373	0.871954325
M07 - M30	0.347148831	0.72847951	0.876873484
M01 - M32	0.350788642	0.725746919	0.877647437
M25 - M39	-0.348917893	0.727150952	0.877984122
M18 - M33	0.347413715	0.728280531	0.877988894
M24 - M25	0.342665125	0.731850403	0.879573673
M21 - M26	-0.340192959	0.73371122	0.880453464
M03 - M24	0.334127208	0.73828357	0.880521689
M01 - M39	0.336721602	0.736326777	0.880881727

M01 - M28	-0.334228224	0.738207348	0.881779068
M02 - M06	-0.336721602	0.736326777	0.882234848
M29 - M37	-0.328075452	0.742854614	0.884620762
M17 - M37	0.320490115	0.748596823	0.890099881
M27 - M34	0.314241651	0.753337511	0.891658966
M09 - M14	-0.306111981	0.759519386	0.892206508
M18 - M39	-0.312040022	0.755010107	0.892284672
M31 - M35	0.315051203	0.752722778	0.89228536
M01 - M05	0.310487585	0.756190199	0.892327315
M22 - M40	0.30696759	0.758868042	0.892785931
M18 - M24	-0.297778591	0.765872156	0.892945114
M02 - M24	0.299249913	0.764749368	0.892970819
M21 - M36	-0.288753454	0.772770054	0.892978729
M03 - M16	0.315622504	0.752289055	0.893128558
M06 - M11	0.30187833	0.762744816	0.893304739
M05 - M25	0.300115634	0.764088954	0.893537308
M13 - M35	-0.307367027	0.758564023	0.893776341
M08 - M34	-0.29520984	0.767833585	0.893895816
M02 - M16	0.30244947	0.762309448	0.894137398
M02 - M07	-0.288774915	0.77275363	0.894284616
M08 - M30	-0.291039337	0.771021235	0.894935362
M30 - M35	-0.289254327	0.772386761	0.895188222
M08 - M11	-0.291858329	0.770394945	0.895541069
M13 - M27	-0.273688937	0.78432368	0.898344303
M08 - M13	-0.272155542	0.785502425	0.898375207
M01 - M14	0.274573857	0.783643653	0.898885367
M16 - M25	0.280472347	0.779115138	0.898979005
M02 - M27	-0.263483587	0.792177872	0.899415925
M02 - M35	-0.266208982	0.790078266	0.899651164
M07 - M31	-0.275199631	0.78316287	0.899656905
M01 - M38	-0.269030146	0.787906484	0.899805355
M02 - M39	0.267194624	0.789319315	0.900100974
M02 - M05	0.263512333	0.792155719	0.900701838
M25 - M26	0.275319973	0.783070421	0.900877475
M05 - M18	0.276349213	0.782279861	0.901297329
M02 - M32	0.257603653	0.79671281	0.901939031
M27 - M30	0.258380912	0.796112947	0.90256991
M27 - M31	-0.252350478	0.800770175	0.903908446
M07 - M11	0.250598023	0.802124911	0.904129234
M16 - M18	0.253047381	0.800231599	0.904609634
M18 - M26	0.246466328	0.805321267	0.905116122
M03 - M39	0.243371569	0.807717566	0.905200721
M20 - M31	0.244684341	0.806700854	0.905362109
M09 - M32	-0.241520319	0.80915187	0.905507114
M03 - M32	0.246738163	0.80511087	0.906185395
M10 - M19	-0.238109094	0.811796482	0.907165123
M06 - M20	-0.234916228	0.814273754	0.908631657

M01 - M08	-0.23151782	0.816912543	0.910273977
M02 - M14	0.222402441	0.824000612	0.915556236
M03 - M05	0.223456501	0.823180234	0.915949476
M10 - M21	0.211227539	0.832709723	0.919990912
M33 - M36	0.214217683	0.830377307	0.920020312
M17 - M22	-0.211931261	0.832160662	0.920688391
M10 - M26	-0.214501827	0.830155743	0.921083185
M11 - M35	-0.204262915	0.838148049	0.924689502
M11 - M27	-0.191968909	0.84776656	0.931349178
M14 - M16	0.19039793	0.848997319	0.931389464
M04 - M20	-0.194419466	0.845847449	0.931865834
M14 - M24	0.192379912	0.847444626	0.932308616
M20 - M29	-0.171779075	0.863611216	0.940805515
M11 - M38	0.177074769	0.85944966	0.941531931
M11 - M28	0.172281031	0.863216596	0.941690832
M09 - M39	-0.174757903	0.861269868	0.94220266
M06 - M35	0.173096828	0.862575318	0.942309171
M08 - M28	-0.14559096	0.884244305	0.947404612
M09 - M24	-0.147097077	0.883055397	0.9474322
M13 - M38	0.140091626	0.888587597	0.948150924
M34 - M38	0.138534962	0.88981764	0.948166338
M02 - M30	-0.136757431	0.891222533	0.94836777
M03 - M14	0.161350086	0.871817677	0.948420904
M06 - M07	0.141309191	0.887625689	0.948421969
M06 - M27	0.147386247	0.882827161	0.948491991
M28 - M34	-0.131985798	0.894995525	0.948500693
M09 - M18	0.142511772	0.886675782	0.948706598
M37 - M40	-0.147837184	0.882471263	0.949417359
M13 - M28	0.132043818	0.894949634	0.949742468
M02 - M13	-0.128962966	0.897386953	0.949744672
M05 - M09	0.148785875	0.881722597	0.949922134
M28 - M30	-0.149835914	0.880894074	0.950342155
M26 - M36	0.132549474	0.894549694	0.950611391
M02 - M34	-0.124790697	0.900689253	0.950659834
M09 - M25	0.15079896	0.880134307	0.950837617
M17 - M40	0.151929791	0.879242311	0.951191405
M01 - M03	0.125488465	0.900136856	0.951364157
M04 - M06	0.118852725	0.905392036	0.951759822
M16 - M32	-0.120261467	0.904276028	0.951869503
M30 - M38	0.155399651	0.876506259	0.952193429
M02 - M11	-0.153683514	0.877859285	0.952336915
M02 - M03	0.152107231	0.879102361	0.952360891
M19 - M36	0.121139001	0.903580938	0.952423151
M05 - M14	-0.109347737	0.912926685	0.95325678
M24 - M32	-0.110617098	0.911919989	0.953482026
M09 - M16	-0.111871537	0.910925265	0.953720412
M10 - M36	-0.112812863	0.91017892	0.954219836

M14 - M39	0.114238758	0.909048528	0.954317432
M04 - M31	0.099110602	0.921050454	0.960453682
M14 - M32	0.089538645	0.928653843	0.965799996
M01 - M02	-0.087209356	0.930505102	0.966436724
M08 - M38	-0.090034708	0.928259634	0.966678925
M19 - M33	-0.070905726	0.943472786	0.97600633
M11 - M34	0.073827172	0.941147906	0.976190647
M02 - M28	-0.072219087	0.942427556	0.976219779
M16 - M39	-0.065955778	0.947413033	0.978784325
M11 - M13	0.055370379	0.955843394	0.98358555
M07 - M35	0.055494302	0.955744669	0.984783147
M06 - M31	-0.047366127	0.962221425	0.984951065
M02 - M38	-0.056593306	0.954869167	0.985182474
M05 - M32	-0.047724979	0.961935427	0.985952211
M24 - M39	-0.048901795	0.960997559	0.986286968
M11 - M30	0.040868613	0.96740064	0.98636928
M05 - M16	0.049678014	0.960378977	0.986950728
M19 - M26	-0.041681069	0.966752946	0.986999081
M32 - M39	0.043106708	0.965616472	0.987130863
M30 - M34	0.034608744	0.972391729	0.990163902
M05 - M24	0.032521894	0.974055856	0.990565278
M28 - M38	0.028181435	0.977517444	0.991500139
M07 - M27	0.029338319	0.976594766	0.991854059
M16 - M24	-0.024839007	0.980183378	0.992913032
M27 - M35	0.015031416	0.988007117	0.995666087
M13 - M34	0.016210787	0.98706623	0.996004734
M13 - M30	-0.016534557	0.986807933	0.997033922
M18 - M25	-0.011648324	0.990706193	0.997097845
M05 - M39	-0.009314628	0.99256811	0.99768444
M02 - M08	-0.017076817	0.986375333	0.997889442
M26 - M33	-0.005982123	0.995226985	0.99906956
M12 - M15	0	1	1
M12 - M23	0	1	1
M15 - M23	0	1	1

Table S5 Results of a Dunn test with fdr correction for multiple testing for developmental time of metamorphs between matrilines. Given are the respective comparisons of matriline number (M1-M40), the z-value, the unadjusted p-value (res.P.unadj) and the adjusted p-value with fdr correction (res.P.adj). Comparisons are sorted from smallest to largest adjusted p-value.

res.Comparison	res.Z	res.P.unadj	res.P.adj
M07 - M24	-5.712408477	1.11E-08	8.69E-06
M24 - M32	5.462582358	4.69E-08	1.83E-05
M07 - M40	-5.24734994	1.54E-07	4.01E-05
M04 - M24	-5.1584234	2.49E-07	4.86E-05
M32 - M40	-5.03740714	4.72E-07	5.26E-05
M11 - M24	-5.064550773	4.09E-07	5.32E-05
M07 - M31	-5.06765951	4.03E-07	6.28E-05
M16 - M24	-4.843023121	1.28E-06	8.31E-05
M11 - M40	-4.846565054	1.26E-06	8.91E-05
M08 - M24	-4.906560465	9.27E-07	9.04E-05
M24 - M29	4.795542503	1.62E-06	9.04E-05
M24 - M33	4.878145351	1.07E-06	9.28E-05
M10 - M24	-4.802724784	1.57E-06	9.39E-05
M31 - M32	4.848070774	1.25E-06	9.72E-05
M04 - M40	-4.702541696	2.57E-06	0.00013361
M07 - M39	-4.676293529	2.92E-06	0.000142402
M11 - M31	-4.65683939	3.21E-06	0.000147329
M16 - M40	-4.610086935	4.03E-06	0.000174417
M29 - M40	-4.566015854	4.97E-06	0.000193862
M33 - M40	-4.574323424	4.78E-06	0.000196134
M07 - M25	-4.484627332	7.30E-06	0.000227889
M07 - M19	-4.467474258	7.91E-06	0.000228651
M04 - M31	-4.498931175	6.83E-06	0.000231612
M08 - M40	-4.51565186	6.31E-06	0.000234454
M10 - M40	-4.505560297	6.62E-06	0.000234702
M32 - M39	-4.486532098	7.24E-06	0.000235273
M07 - M38	-4.469268622	7.85E-06	0.000235463
M11 - M39	-4.443387073	8.86E-06	0.000246685
M16 - M31	-4.410171078	1.03E-05	0.000277812
M05 - M24	-4.361617273	1.29E-05	0.000314692
M29 - M31	-4.364770923	1.27E-05	0.000320193
M31 - M33	4.367433715	1.26E-05	0.000326859
M11 - M19	-4.326482344	1.52E-05	0.000358113
M07 - M35	-4.273243206	1.93E-05	0.000375668
M19 - M32	4.29470385	1.75E-05	0.000379008
M11 - M38	-4.276561649	1.90E-05	0.000379604
M32 - M38	-4.281855375	1.85E-05	0.000380438
M08 - M31	-4.303206524	1.68E-05	0.0003862
M25 - M32	4.284278909	1.83E-05	0.000386485
M10 - M31	-4.29611769	1.74E-05	0.00038736
M11 - M25	-4.243579888	2.20E-05	0.000418502

M05 - M40	-4.218774392	2.46E-05	0.000456177
M16 - M39	-4.187340514	2.82E-05	0.000511974
M07 - M18	-4.143210123	3.42E-05	0.000593628
M29 - M39	-4.144872558	3.40E-05	0.000602732
M04 - M39	-4.125480757	3.70E-05	0.000627325
M07 - M30	-4.108374848	3.98E-05	0.000647486
M11 - M18	-4.111064996	3.94E-05	0.000653604
M33 - M39	-4.094496051	4.23E-05	0.000673486
M16 - M19	-4.067948886	4.74E-05	0.000739889
M19 - M29	4.027457278	5.64E-05	0.000814426
M10 - M39	-4.028745925	5.61E-05	0.000825257
M05 - M31	-4.019132226	5.84E-05	0.000828401
M17 - M24	-4.036671873	5.42E-05	0.000829168
M32 - M35	-4.030788388	5.56E-05	0.000833851
M16 - M38	-4.012372533	6.01E-05	0.000837268
M18 - M32	3.990229873	6.60E-05	0.000903285
M07 - M20	-3.984050526	6.78E-05	0.000911127
M08 - M39	-3.974220136	7.06E-05	0.000917933
M29 - M38	-3.969904577	7.19E-05	0.000919395
M16 - M25	-3.975463365	7.02E-05	0.000928628
M19 - M33	3.95087377	7.79E-05	0.00096406
M07 - M28	-3.951607913	7.76E-05	0.000976608
M11 - M35	-3.926222993	8.63E-05	0.001004572
M11 - M20	-3.928259883	8.56E-05	0.001011196
M25 - M29	3.931392284	8.45E-05	0.001013464
M04 - M19	-3.934618841	8.33E-05	0.00101557
M30 - M32	3.901927618	9.54E-05	0.001033822
M04 - M38	-3.909765743	9.24E-05	0.00104436
M33 - M38	-3.902828231	9.51E-05	0.001044488
M11 - M30	-3.911063825	9.19E-05	0.001054038
M04 - M25	-3.903991216	9.46E-05	0.001054328
M10 - M19	-3.888745741	0.000100764	0.001076652
M25 - M33	3.873717973	0.000107187	0.001129814
M05 - M39	-3.868340978	0.000109578	0.001139615
M16 - M18	-3.84934873	0.000118432	0.001215489
M10 - M38	-3.837078105	0.000124507	0.001261239
M10 - M25	-3.804954845	0.00014183	0.001349115
M07 - M37	-3.810889029	0.000138468	0.001350062
M20 - M32	3.816100016	0.000135578	0.001355776
M08 - M19	-3.806046406	0.000141206	0.001359761
M18 - M29	3.811364235	0.000138202	0.001364526
M01 - M07	3.795935186	0.000147088	0.001365816
M07 - M27	-3.797945137	0.000145901	0.001371114
M05 - M19	-3.790443155	0.000150379	0.001379946
M11 - M23	-3.786761995	0.000152623	0.001384257
M08 - M38	-3.7650934	0.000166487	0.001492641
M17 - M40	-3.758054697	0.000171239	0.001517804

M08 - M25	-3.744310645	0.00018089	0.001585326
M01 - M11	3.733974105	0.000188482	0.001615559
M11 - M27	-3.735593769	0.000187273	0.001623031
M28 - M32	3.721673524	0.000197907	0.001677905
M11 - M28	-3.709870031	0.000207366	0.001702581
M11 - M37	-3.710942992	0.000206489	0.001713416
M05 - M38	-3.711844858	0.000205754	0.001725679
M18 - M33	3.702907783	0.000213142	0.001731782
M07 - M23	-3.650376817	0.000261856	0.002063107
M05 - M25	-3.655466414	0.000256715	0.002064305
M16 - M20	-3.65201909	0.000260187	0.002070872
M10 - M18	-3.645241108	0.000267141	0.002083702
M16 - M35	-3.633698925	0.000279387	0.002136488
M04 - M18	-3.633964513	0.000279099	0.00215542
M05 - M18	-3.626305362	0.000287505	0.002177225
M01 - M32	3.616159239	0.000299006	0.002179673
M27 - M32	3.618146392	0.000296721	0.002183415
M16 - M30	-3.622932386	0.000291282	0.002184615
M04 - M35	-3.610244591	0.000305908	0.002189069
M32 - M37	-3.619015639	0.000295726	0.00219682
M20 - M29	3.611527483	0.000304399	0.002198436
M29 - M35	-3.584661153	0.000337516	0.002393296
M29 - M30	-3.577532231	0.000346853	0.002437349
M23 - M32	3.544113398	0.000393936	0.002719203
M16 - M23	-3.545869196	0.00039132	0.002725267
M08 - M18	-3.530836092	0.000414248	0.002809685
M33 - M35	-3.531570443	0.0004131	0.002826472
M23 - M29	3.515839816	0.000438365	0.002922436
M17 - M31	-3.513346598	0.0004425	0.002924999
M14 - M24	-3.517141423	0.000436221	0.002933212
M20 - M33	3.499331942	0.000466426	0.003057243
M30 - M33	3.492466828	0.000478581	0.003110777
M04 - M30	-3.490166788	0.000482719	0.003111743
M10 - M35	-3.452984011	0.000554422	0.003544664
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M16 - M27	-3.445000634	0.000571058	0.003621344
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M10 - M20	-3.437203913	0.000587753	0.003667578
M05 - M23	-3.434808324	0.000592973	0.003670786
M10 - M30	-3.421150804	0.000623567	0.003741405
M07 - M36	-3.422084384	0.00062143	0.003757485
M16 - M37	-3.414879211	0.000638103	0.003770609
M05 - M20	-3.414995418	0.000637831	0.003797771
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M16 - M28	-3.404625305	0.000662549	0.003856628
M27 - M29	3.402532677	0.000667644	0.003857497

M08 - M35	-3.405689828	0.000659971	0.003870507
M29 - M37	-3.37080813	0.00074948	0.004236193
M23 - M33	3.371492339	0.000747621	0.004256529
M28 - M29	3.356314059	0.000789888	0.004432466
M08 - M30	-3.332078392	0.000862	0.00480257
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M05 - M30	-3.324852959	0.000884652	0.004859355
M08 - M20	-3.318325489	0.000905589	0.004939576
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M27 - M33	3.281303455	0.001033285	0.005482737
M17 - M39	-3.271311826	0.001070498	0.00556659
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M33 - M37	-3.254849824	0.001134523	0.005821896
M32 - M36	-3.246548847	0.001168134	0.005955193
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M06 - M24	-3.221108612	0.001276957	0.006425979
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M16 - M36	-3.127340319	0.001763956	0.007999336
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M05 - M28	-3.095450941	0.001965139	0.008809243
M29 - M36	-3.084872363	0.002036395	0.008973945
M08 - M27	-3.086954274	0.002022187	0.009013176
M01 - M08	3.084923917	0.002036042	0.009023369
M17 - M38	-3.066635104	0.00216483	0.009486332
M08 - M37	-3.062959237	0.002191599	0.009549985
M13 - M24	-3.050247588	0.002286528	0.009908287
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M14 - M31	-3.038300718	0.002379164	0.010196417

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M17 - M25	-3.004926466	0.002656451	0.011261041
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M16 - M21	-2.996976661	0.002726716	0.011496424
M24 - M34	2.99387394	0.002754597	0.011551537
M21 - M29	2.958992166	0.003086469	0.012805565
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M17 - M18	-2.941263139	0.003268767	0.01341915
M33 - M36	-2.933324015	0.003353537	0.013695073
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M13 - M40	-2.919043777	0.003511069	0.01411667
M05 - M36	-2.920247981	0.00349753	0.014135094
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M11 - M34	-2.878332403	0.003997836	0.015749053
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M07 - M34	-2.871167986	0.004089581	0.016029515
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M05 - M21	-2.848199679	0.004396733	0.016811037
M26 - M29	2.849187579	0.004383103	0.01684148
M22 - M32	2.850418661	0.004366172	0.016859475
M06 - M19	-2.851898606	0.004345896	0.016864671
M14 - M39	-2.845171547	0.004438751	0.016888907
M16 - M22	-2.839720851	0.004515303	0.017096777
M26 - M32	2.827483776	0.004691539	0.017678264
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M17 - M23	-2.754479927	0.00587855	0.02113027
M14 - M19	-2.758434672	0.005807891	0.021168949
M06 - M23	-2.754668685	0.00587516	0.021215854
M06 - M18	-2.755058659	0.005868161	0.021289144
M06 - M38	-2.737933366	0.00618266	0.022121445
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M26 - M33	2.675196787	0.00746854	0.025890938
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M32 - M34	-2.64739484	0.008111458	0.027749726
M14 - M38	-2.643135926	0.008214205	0.027978516

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M15 - M24	-2.62093016	0.008769022	0.029355526
M22 - M33	2.618393471	0.008834487	0.02944829
M15 - M40	-2.611742939	0.009008196	0.029899544
M14 - M18	-2.580353039	0.009869936	0.032620974
M17 - M30	-2.567203442	0.010252244	0.033741563
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M16 - M34	-2.515735248	0.011878437	0.037972054
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M03 - M39	-2.494162797	0.012625462	0.039869881
M14 - M23	-2.487963716	0.012847684	0.040245757
M12 - M24	-2.487984427	0.012846936	0.040405685
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M13 - M19	-2.471897623	0.0134398	0.04159938
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M08 - M26	-2.461618833	0.013831157	0.042141807
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M03 - M19	-2.438700982	0.014740159	0.044051051
M15 - M23	-2.424427461	0.015332546	0.045646511
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M15 - M19	-2.381496307	0.017242464	0.049996735
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M34 - M39	-2.382596021	0.017191048	0.050221039
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M09 - M19	-2.352117713	0.018666867	0.053333904
M17 - M37	-2.339663196	0.019301137	0.054944843
M13 - M38	-2.337653992	0.019405205	0.055040217
M13 - M18	-2.336285768	0.019476353	0.055041867

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M33 - M34	-2.264753775	0.023527794	0.062633717
M17 - M28	-2.257653886	0.023967246	0.062733061
M12 - M39	-2.25820938	0.023932609	0.062853316
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M12 - M19	-2.258412475	0.023919956	0.063032316
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M09 - M38	-2.214405047	0.026800928	0.068992488
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M03 - M25	-2.200049394	0.027803391	0.070871388
M07 - M14	-2.18407834	0.028956492	0.073331376
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M34 - M38	-2.177919298	0.029412045	0.074243998
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M09 - M25	-2.111022352	0.034770391	0.085825648
M14 - M30	-2.106827337	0.035132549	0.08644602
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M04 - M34	-2.100388561	0.035694674	0.087278514
M03 - M16	2.092838915	0.036363537	0.088636121
M15 - M20	-2.080468421	0.037482589	0.091079189
M03 - M32	2.074999746	0.037986558	0.092017129
M07 - M13	-2.071969517	0.038268284	0.092412574

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M13 - M20	-2.030109469	0.042345413	0.099486212
M17 - M36	-2.031328576	0.042221674	0.099495184
M06 - M36	-2.015307589	0.04387243	0.102764251
M14 - M35	-2.013169638	0.044096786	0.102980519
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M01 - M14	1.986029777	0.047030018	0.10853081
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M14 - M32	1.977750134	0.047956902	0.109696138
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M09 - M20	-1.953895252	0.050713606	0.114325471
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M02 - M15	1.796958102	0.072342272	0.155446203
M03 - M33	1.789226972	0.07357827	0.157667721
M15 - M37	-1.782401845	0.074683725	0.159598097
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M14 - M26	-1.775282496	0.075851254	0.16120975
M24 - M28	1.769187392	0.076862608	0.16291531
M15 - M35	-1.762441393	0.07799475	0.164866952
M14 - M28	-1.755857732	0.079112704	0.166328596

M03 - M30	-1.756661701	0.078975489	0.166488869
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M01 - M13	1.729291912	0.083756863	0.172831623
M13 - M27	-1.731107918	0.083432518	0.173078097
M01 - M12	1.732094641	0.083256713	0.173173963
M07 - M17	-1.732371246	0.083207484	0.173534325
M12 - M27	-1.733295816	0.083043104	0.173655821
M09 - M30	-1.720702929	0.085304745	0.175561216
M03 - M10	1.715716096	0.086214017	0.176965614
M02 - M12	1.712875838	0.086735384	0.177568503
M15 - M21	-1.69022467	0.09098498	0.18578085
M03 - M27	-1.680970201	0.092768703	0.188928429
M01 - M03	1.679109348	0.093130735	0.189171805
M13 - M35	-1.671172831	0.094687545	0.190843114
M09 - M27	-1.673437167	0.094241266	0.190930358
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M13 - M26	-1.640341586	0.100934164	0.20135204
M13 - M37	-1.63423408	0.102209709	0.201831831
M15 - M36	-1.63479945	0.102091096	0.202109277
M13 - M33	1.635054423	0.102037639	0.202517452
M15 - M28	-1.635410644	0.101962993	0.202885547
M16 - M17	-1.627019442	0.103732969	0.203295768
M14 - M21	-1.627372094	0.103658095	0.20366074
M12 - M35	-1.627609811	0.103607647	0.204075669
M14 - M36	-1.621188751	0.104977164	0.205218517
M03 - M35	-1.618806353	0.105488935	0.205703423
M02 - M14	1.610826554	0.107217532	0.207517803
M09 - M16	1.611242057	0.107126974	0.207858308
M07 - M09	-1.612111652	0.106937646	0.208008388
M24 - M35	1.597659487	0.11011878	0.212605565
M03 - M26	-1.596252327	0.110432469	0.212684755
M30 - M34	1.591091951	0.111588884	0.214382584
M10 - M14	-1.589151096	0.112026284	0.214694108
M09 - M35	-1.580328247	0.114031714	0.217468795
M03 - M37	-1.581181245	0.113836602	0.217628799
M17 - M29	1.577340626	0.114717169	0.217711416
M09 - M37	-1.578385455	0.114477085	0.217785673
M09 - M29	1.57075045	0.116240626	0.217951173
M35 - M40	-1.572695984	0.11578923	0.218153622
M12 - M21	-1.571314392	0.11610964	0.218230167
M05 - M09	-1.572845308	0.115754642	0.218616514
M15 - M22	-1.57301311	0.115715783	0.219073569
M10 - M13	-1.563738398	0.117879016	0.220493124

M02 - M09	1.548158181	0.121584217	0.22687964
M05 - M17	-1.543366527	0.12274187	0.22849322
M17 - M32	1.529118641	0.126235035	0.234436493
M02 - M13	1.525003919	0.12725812	0.235216431
M03 - M04	1.525743589	0.127073734	0.235433522
M23 - M28	1.518496181	0.128889362	0.237668326
M27 - M34	1.514210315	0.129972506	0.239100365
M01 - M34	1.512223162	0.1304771	0.239463855
M12 - M36	-1.508676054	0.131381588	0.23999447
M04 - M11	1.509418723	0.131191811	0.240210359
M12 - M28	-1.501446155	0.133240204	0.242820932
M08 - M11	1.492790656	0.135491994	0.24634908
M02 - M03	1.488061813	0.136734593	0.248030192
M09 - M32	1.467810177	0.142155792	0.257265703
M26 - M34	1.460374959	0.144187051	0.260337731
M12 - M22	-1.454102832	0.145917799	0.262854233
M14 - M22	-1.451554754	0.146625447	0.263520389
M13 - M28	-1.450190649	0.147005362	0.263595821
M34 - M35	-1.436881785	0.150751586	0.269076058
M13 - M21	-1.437806717	0.150488903	0.269223267
M03 - M08	1.435337655	0.151190902	0.269244073
M09 - M21	-1.429420764	0.152883334	0.271637814
M23 - M35	1.41187928	0.15798551	0.280065221
M28 - M31	-1.407161275	0.159379572	0.281895841
M34 - M37	-1.404046201	0.160305092	0.282891339
M09 - M28	-1.394722286	0.163099602	0.287173114
M13 - M36	-1.391514757	0.164069388	0.288230006
M03 - M28	-1.390292077	0.164440203	0.288232266
M03 - M21	-1.383728193	0.166441697	0.291086377
M04 - M14	-1.381340798	0.1671742	0.291713368
M02 - M34	1.377926049	0.168226121	0.292893693
M09 - M36	-1.370559941	0.170512178	0.296212692
M36 - M40	-1.364692511	0.172349715	0.298739505
M19 - M28	1.363380212	0.172762714	0.29879139
M28 - M39	-1.358863609	0.174189812	0.300593039
M04 - M13	-1.348724048	0.177425618	0.305501064
M24 - M36	1.34655035	0.178125094	0.306029897
M06 - M34	-1.344397067	0.178820022	0.306548609
M23 - M36	1.334505649	0.182038197	0.311381126
M03 - M36	-1.332990761	0.182534836	0.311547422
M18 - M28	1.296222945	0.194898687	0.331923528
M08 - M14	-1.283622703	0.199274002	0.337899395
M09 - M33	1.283822974	0.199203903	0.338516436
M09 - M22	-1.278101291	0.201213714	0.340448366
M13 - M22	-1.272044539	0.203357295	0.343330498
M08 - M13	-1.270531303	0.20389544	0.343495557
M17 - M33	1.253234894	0.210120235	0.35321936

M37 - M40	-1.244011641	0.213495279	0.358121113
M23 - M37	1.241390461	0.214461541	0.358969962
M31 - M35	1.237667054	0.21583954	0.360502872
M22 - M23	-1.226474585	0.220020134	0.366700223
M09 - M10	1.221694945	0.221823009	0.368916731
M24 - M37	1.219676173	0.222587658	0.369400794
M19 - M35	1.214404043	0.224593472	0.37115023
M03 - M22	-1.214808357	0.224439193	0.37168274
M21 - M34	1.211853455	0.225568482	0.371973395
M35 - M39	-1.201425421	0.22958622	0.377800109
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M23 - M30	1.187223972	0.235139288	0.386123462
M30 - M40	-1.175170438	0.239926535	0.392332698
M10 - M17	-1.173320147	0.240667446	0.392720937
M22 - M40	-1.167522845	0.242999288	0.395698215
M28 - M38	-1.159821273	0.246121581	0.399947569
M18 - M35	1.156827136	0.247342993	0.401096745
M24 - M30	1.147525783	0.251164383	0.406448587
M34 - M36	-1.142612771	0.253199401	0.408893443
M22 - M24	-1.128947686	0.258919903	0.417267612
M17 - M34	-1.118276199	0.263449049	0.423691254
M21 - M23	-1.109263025	0.267316731	0.427268546
M23 - M27	1.109885887	0.267048203	0.427715808
M01 - M23	-1.111087062	0.266530876	0.427765604
M19 - M36	1.086146888	0.277414011	0.442500877
M03 - M06	1.083734767	0.278482416	0.443298539
M31 - M36	1.066903648	0.286015348	0.454362467
M36 - M39	-1.060000195	0.289144511	0.458399834
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M01 - M40	-1.036803666	0.299827367	0.472455244
M27 - M40	-1.035040823	0.300649847	0.47279613
M04 - M16	1.037019343	0.299726842	0.473252909
M08 - M16	1.030609043	0.302724194	0.474146327
M04 - M05	1.027176615	0.304337308	0.475717635
M05 - M08	-1.025840428	0.304966806	0.475748218
M10 - M11	1.017024117	0.309141957	0.481298855
M15 - M34	-1.014902491	0.31015231	0.481909964
M11 - M12	-1.012100763	0.311489877	0.483026052
M21 - M40	-1.005571801	0.314621594	0.486914372
M25 - M28	1.000697182	0.31697323	0.489582415
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M01 - M24	-0.993294546	0.320566435	0.491241295
M24 - M27	0.991395321	0.321492584	0.49169454
M06 - M13	-0.994875359	0.319796889	0.491995213
M04 - M07	0.99330675	0.320560489	0.492199176
M04 - M29	0.98466133	0.324790453	0.49576625

M08 - M29	0.979850127	0.327160104	0.498407972
M07 - M08	-0.974315157	0.329900081	0.500626582
M04 - M09	-0.974935624	0.329592194	0.50113433
M19 - M37	0.962192043	0.335953145	0.508822239
M21 - M24	-0.957367531	0.338381781	0.511507343
M11 - M33	-0.954896087	0.339630249	0.512401536
M06 - M14	-0.951891446	0.341152038	0.513703841
M19 - M22	0.939496556	0.34747586	0.522218055
M18 - M37	0.93314649	0.350744339	0.526116508
M37 - M39	-0.930529402	0.352097048	0.526122026
M31 - M37	0.931121567	0.351790684	0.526673193
M08 - M09	-0.9253023	0.354808683	0.529160177
M18 - M22	0.92165994	0.356705982	0.530974554
M11 - M32	-0.905080795	0.365422586	0.541881401
M02 - M28	0.903490507	0.366265624	0.542100924
M22 - M39	-0.905550362	0.365173892	0.542544068
M06 - M11	0.894945014	0.370816482	0.545729918
M22 - M31	-0.896076701	0.3702118	0.545869951
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M19 - M30	0.888737385	0.374144234	0.548557335
M11 - M15	-0.889016932	0.37399398	0.549369688
M36 - M38	-0.885032214	0.376139257	0.550447693
M04 - M17	-0.881775326	0.377898317	0.551986306
M12 - M34	-0.879323914	0.379225673	0.552889766
M18 - M30	0.862443795	0.388443349	0.565272038
M23 - M25	0.852407471	0.393987975	0.571209332
M30 - M39	-0.853522918	0.393369384	0.571374524
M30 - M31	-0.850314971	0.395149995	0.571831162
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M02 - M35	0.821196456	0.411534375	0.593339764
M20 - M23	-0.817276642	0.413770352	0.595462869
M06 - M09	-0.814904913	0.415126741	0.596314655
M26 - M28	0.810398168	0.417711371	0.598924392
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M08 - M17	-0.805370401	0.420605967	0.600865667
M02 - M36	0.801766741	0.422687901	0.602735946
M19 - M27	0.783269661	0.433468775	0.612510226
M04 - M32	0.784543082	0.432721513	0.612564029
M19 - M21	0.788177083	0.430593132	0.612888035
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M18 - M21	0.778105683	0.436506713	0.615687588
M01 - M18	-0.76956587	0.441557457	0.620567237
M18 - M27	0.76804649	0.442459565	0.620716656
M05 - M12	-0.754230908	0.450710581	0.631156649
M21 - M39	-0.748294553	0.454282509	0.63162274

M37 - M38	-0.748956547	0.453883396	0.632194731
M22 - M38	-0.749054243	0.453824513	0.633243506
M02 - M22	0.750018259	0.453243708	0.633566473
M01 - M39	-0.744038598	0.456853124	0.634066614
M20 - M35	0.741245253	0.45854475	0.634157632
M27 - M39	-0.74233988	0.457881435	0.634365043
M13 - M15	0.737828064	0.460618937	0.635898709
M01 - M31	-0.729126493	0.46592429	0.63982561
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M27 - M31	-0.727310487	0.467035776	0.640224789
M21 - M31	-0.730314523	0.465197953	0.641085518
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M23 - M38	0.708693369	0.478514781	0.653662923
M12 - M16	0.702687493	0.482250524	0.65761435
M26 - M35	0.699198061	0.484428262	0.659431142
M03 - M17	0.691859893	0.48902532	0.66452918
M14 - M15	0.686356958	0.49248804	0.668070732
M03 - M12	0.677058558	0.498368801	0.674874418
M02 - M30	0.674080859	0.500259908	0.676261227
M12 - M29	0.672658113	0.501164821	0.676312389
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M02 - M21	0.659226575	0.509750284	0.680830859
M06 - M17	-0.659595547	0.509513413	0.681681753
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M23 - M31	0.649437565	0.51605559	0.68807412
M05 - M15	-0.63532063	0.525219322	0.69909739
M02 - M27	0.631970161	0.527406376	0.699620702
M01 - M02	-0.632878164	0.526813208	0.700024366
M05 - M10	-0.621820847	0.534059677	0.707243714
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M05 - M06	-0.602247733	0.547009247	0.717087752
M09 - M15	0.603462443	0.546201093	0.717233759
M12 - M13	-0.607568381	0.543473777	0.717275036
M20 - M40	-0.603559437	0.546136588	0.718358412
M22 - M25	-0.604214867	0.545700798	0.718997673
M22 - M26	-0.597948828	0.549874078	0.719633861
M21 - M38	-0.591798433	0.553985573	0.723800246
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M25 - M37	0.583474486	0.559573928	0.727446106
M07 - M12	-0.579389588	0.562326328	0.72980788
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M27 - M38	-0.567371899	0.570461538	0.736688741
M04 - M33	0.558642753	0.576405556	0.73704317
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M08 - M33	0.565078913	0.572020091	0.737480448
M13 - M17	0.558695393	0.576369624	0.7382074
M12 - M14	-0.551525376	0.581273576	0.740838872
M15 - M29	0.546534717	0.58469843	0.743988215
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M19 - M23	-0.516248756	0.605680681	0.764451345
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M01 - M28	0.512450568	0.608335707	0.765325567
M23 - M24	0.508992234	0.610757668	0.765901898
M09 - M34	-0.505441432	0.613248825	0.767791466
M06 - M29	0.503480148	0.614626734	0.768283418
M26 - M30	0.498708502	0.617984754	0.771244973
M11 - M29	-0.491406152	0.623139214	0.771505693
M28 - M30	-0.492175451	0.622595317	0.772057786
M16 - M33	-0.49250566	0.622361921	0.772997291
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M05 - M32	-0.494399793	0.62102386	0.773799698
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M18 - M23	-0.473942395	0.635540986	0.779437058
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M38 - M40	-0.446251177	0.655415819	0.798788029
M15 - M17	-0.437427145	0.661801601	0.799079333
M20 - M30	0.446949231	0.654911717	0.799422753
M18 - M25	0.435557776	0.663157612	0.799479037
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M02 - M25	0.430135504	0.667097075	0.80298722
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M13 - M34	-0.417416098	0.676374082	0.810402126
M16 - M32	-0.411799171	0.680486629	0.811589558

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M20 - M21	0.412729346	0.679804922	0.813263557
M18 - M20	0.40746606	0.683665717	0.814136274
M12 - M33	0.401885902	0.687767999	0.816528218
M25 - M27	0.400417253	0.688849219	0.816568983
M19 - M20	0.398222461	0.690466211	0.81724377
M01 - M25	-0.402180096	0.68755149	0.817515491
M06 - M07	0.384729756	0.700437647	0.827789946
M05 - M07	-0.379587292	0.704251795	0.831038427
M01 - M20	-0.36895953	0.71215789	0.836571015
M20 - M27	0.367339866	0.713365521	0.836729483
M25 - M39	-0.369945248	0.711423286	0.836968572
M07 - M10	-0.370891301	0.710718494	0.837402456
M29 - M32	-0.362120355	0.717262093	0.840036686
M31 - M40	-0.353688131	0.723572618	0.841112731
M15 - M32	0.352206326	0.724683533	0.84115053
M28 - M37	-0.355641867	0.722108786	0.841920557
M24 - M38	0.357054254	0.72105119	0.841946001
M21 - M28	0.353890166	0.723421197	0.842191841
M10 - M12	-0.357557176	0.720674731	0.842768051
M27 - M35	0.344245161	0.730661922	0.846829567
M01 - M35	0.34228365	0.732137438	0.847280715
M20 - M39	-0.340453438	0.733515081	0.847617427
M09 - M17	0.328072434	0.742856896	0.854614128
M25 - M31	-0.328511708	0.742524793	0.855493853
M02 - M38	0.328697124	0.742384629	0.856597648
M03 - M34	-0.319658988	0.749226852	0.860672967
M27 - M36	0.317660315	0.750742617	0.861145943
M01 - M36	0.315961597	0.752031657	0.861357846
M30 - M35	0.306193672	0.75945719	0.868587402
M12 - M17	-0.301848568	0.762767504	0.871096125
M20 - M31	-0.298826333	0.765072557	0.872451162
M05 - M11	0.288603517	0.772884804	0.878790301
M07 - M33	-0.289782741	0.771982452	0.879045712
M06 - M32	0.27679456	0.781937859	0.88521267
M07 - M16	0.278234584	0.780832289	0.885245909
M30 - M36	0.279664956	0.779734567	0.885288154
M02 - M31	0.273238538	0.784669858	0.8870181
M15 - M33	0.27015431	0.787041541	0.88841158
M26 - M40	-0.266836041	0.789595403	0.89000638
M39 - M40	-0.264678322	0.791257278	0.890592608
M18 - M38	0.260573636	0.794421323	0.891580765
M02 - M26	0.261856194	0.793432314	0.891753897
M24 - M31	0.251215104	0.801647808	0.898398405
M03 - M14	0.245026563	0.806435864	0.902467681
M22 - M27	-0.241581389	0.809104545	0.90286344
M01 - M22	0.240062009	0.810282186	0.902885864

M19 - M38	0.242301781	0.808546334	0.903533152
M02 - M39	0.235172804	0.814074613	0.905817687
M07 - M29	0.22798582	0.819657264	0.909434803
M10 - M15	-0.225825585	0.821337077	0.910004148
M03 - M09	0.228349813	0.819374302	0.910415891
M06 - M12	-0.211649661	0.832380363	0.91962703
M28 - M35	-0.213061017	0.831279371	0.919713346
M10 - M32	0.209819706	0.833808392	0.919901762
M21 - M35	0.203846191	0.838473695	0.923742206
M22 - M30	-0.201797434	0.840075088	0.924201084
M21 - M36	0.199798444	0.841638218	0.924616634
M24 - M26	0.193720925	0.846394407	0.928533948
M20 - M26	-0.166690675	0.867613443	0.929585832
M27 - M37	0.160166901	0.872749602	0.929979084
M01 - M37	0.158404058	0.874138416	0.930188219
M02 - M19	0.190162386	0.849181885	0.930283526
M19 - M40	-0.173430019	0.862313431	0.930296647
M05 - M29	-0.161054259	0.872050666	0.930505498
M02 - M18	0.167109331	0.867284024	0.930511057
M25 - M38	-0.188372393	0.850584739	0.93051346
M18 - M31	0.168164528	0.866453846	0.930900826
M04 - M06	0.169165185	0.865666713	0.931337981
M24 - M39	0.161434104	0.871751508	0.931460515
M25 - M26	-0.173522062	0.862241088	0.931506993
M02 - M24	0.162709771	0.870746953	0.931663407
M06 - M08	-0.185464978	0.852864347	0.931700547
M18 - M26	0.154583362	0.877149788	0.932121028
M28 - M36	-0.153014894	0.87838653	0.932165297
M36 - M37	-0.169484787	0.865415338	0.932353541
M22 - M28	0.180037327	0.857123264	0.93243535
M06 - M33	0.181439757	0.856022415	0.932538385
M20 - M38	-0.173628014	0.862157814	0.93270887
M02 - M23	-0.182578631	0.855128653	0.932867621
M35 - M37	-0.174547601	0.861435124	0.933221385
M38 - M39	-0.174967981	0.861104793	0.934160972
M07 - M32	-0.175193041	0.860927952	0.93526992
M19 - M31	0.142961821	0.886320334	0.938032375
M21 - M22	0.143554257	0.885852464	0.938811035
M09 - M13	-0.137542691	0.890601851	0.941286509
M06 - M10	0.129459719	0.896993896	0.941662502
M18 - M40	-0.127750202	0.89834666	0.941815047
M19 - M26	0.13433721	0.89313593	0.94268745
M13 - M14	0.129460833	0.896993015	0.942930662
M24 - M40	-0.129643901	0.896848168	0.944050704
M05 - M16	-0.123069764	0.902051854	0.944430129
M31 - M38	0.120764413	0.903877638	0.945073134
M32 - M33	-0.129904959	0.896641621	0.945108736

M04 - M12	-0.118528503	0.905648912	0.945657499
M30 - M37	0.10963926	0.912695473	0.951741269
M06 - M15	-0.098841466	0.921264145	0.95176958
M02 - M40	0.099866927	0.920449974	0.952189628
M03 - M13	0.100750705	0.91974836	0.952727385
M22 - M37	-0.106626153	0.91508557	0.952959605
M12 - M15	0.102979322	0.917979383	0.953427321
M18 - M39	0.104077516	0.917107833	0.953792147
M08 - M12	-0.100841165	0.919676548	0.953919824
M26 - M39	-0.081679914	0.934901252	0.962035589
M01 - M21	0.082806199	0.93400564	0.962383618
M21 - M27	-0.084325579	0.932797576	0.962410197
M19 - M39	0.075476357	0.939835708	0.965839068
M10 - M33	0.073510876	0.94139959	0.966173263
M19 - M24	-0.068759281	0.945181231	0.968779711
M31 - M39	-0.066284227	0.947151541	0.969525199
M27 - M30	0.059928205	0.952212816	0.973428567
M01 - M30	0.058112199	0.953659258	0.973631179
M21 - M37	0.055324891	0.955879633	0.974622371
M04 - M08	-0.035329506	0.971816995	0.980617408
M08 - M15	0.035958865	0.971315159	0.981380601
M26 - M38	0.042041132	0.966465908	0.981566938
M18 - M19	0.032018323	0.974457438	0.982011372
M16 - M29	-0.042467957	0.966125656	0.982500667
M21 - M30	-0.036035256	0.971254247	0.982591845
M26 - M31	-0.039077905	0.968828277	0.982686679
M18 - M24	-0.027353358	0.978177899	0.983220053
M09 - M14	-0.036297617	0.97104505	0.983656024
M22 - M36	0.042542634	0.966066125	0.983722686
M22 - M35	0.028028851	0.97763914	0.983946489
M35 - M36	0.022557375	0.982003345	0.985794863
M04 - M15	0.019997779	0.984045144	0.986574823
M20 - M25	-0.000574272	0.999541798	0.999541798
M01 - M27	-0.001698718	0.99864462	0.999926577

Appendix S2 **Summary of additional linear model**

Instead of number of fathers we used the effective mating frequency (me) as predictor variable. As seen from the model output none of the variables had a significant influence on mean SVL per matriline.

```
Call:
lm(formula = av_size ~ n + me + av_days, data = g_new_all)

Residuals:
     Min       1Q   Median       3Q      Max
-0.88133 -0.23692  0.01119  0.27032  0.86698

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 13.444997   0.877656  15.319   <2e-16 ***
n            0.020688   0.012594   1.643    0.109
me          -0.168052   0.116968  -1.437    0.159
av_days      0.005687   0.007940   0.716    0.478
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.4399 on 36 degrees of freedom
Multiple R-squared:  0.09253,  Adjusted R-squared:  0.01691
F-statistic: 1.224 on 3 and 36 DF,  p-value: 0.3152
```

Appendix S3 **Summary of GAM**

The response variable, mean BCI, was modelled with a generalized additive model. The mean developmental team was used without smoother as it showed a linear relationship with BCI. The number off offspring and number of fathers were used with a cubic regression spline (cs). The number of fathers ranged from 1 to 5, therefore, we reduced number of knots to 3.

```
Family: gaussian
Link function: identity

Formula:
av_bci ~ av_days + s(n, bs = "cs") + s(n_fathers, bs = "cs",
    k = 3)

Parametric coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.1463089  0.0295109   4.958 1.51e-05 ***
av_days     0.0004605  0.0002689   1.712    0.095 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
                edf Ref.df F p-value
s(n)         5.433e-05      9 0   0.435
s(n_fathers) 4.678e-05      2 0   0.643

R-sq.(adj) =  0.0472   Deviance explained = 7.16%
-REML = -98.348  Scale est. = 0.00024243  n = 40
```

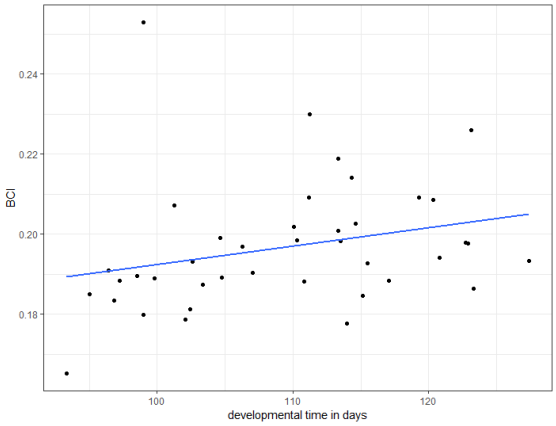

Figure S5 Results of the GAM. The developmental time showed a positive linear trend on the BCI, but was not significant.

7 Declaration of independance / Eigenständigkeitserklärung

I hereby certify that I have written the present work independently and have used no other than the specified tools. The parts of the work taken from other works, either verbally or in terms of content, have been identified by corresponding information from the sources. The underlying doctorate regulations are known to me, the work corresponds to the principles of the Humboldt University in Berlin to ensure good scientific practice.

This work did not exist in the same or similar form to any examining authority.

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe. Die Stellen der Arbeit, die anderen Werken wörtlich oder inhaltlich entnommen sind, wurden durch entsprechende Angaben der Quellen kenntlich gemacht. Die zugrunde liegende Promotionsordnung ist mir bekannt, die Arbeit entspricht den Grundsätzen der Humboldt-Universität zu Berlin zur Sicherung guter wissenschaftlicher Praxis.

Diese Arbeit hat in gleicher oder ähnlicher Form noch keiner Prüfungsbehörde vorgelegen.

Carolin Dittrich, Berlin, den 19. Dezember 2019